

The case for staying in UNESCO

This month's biannual meeting of the general assembly of UNESCO at Sofia is not the make-or-break meeting it might have been, but a sign to reluctant members that they had best stay in.

OF all international organizations, UNESCO (United Nations Educational, Scientific and Cultural Organization) is the most high-minded and thus the most deserving of support. Founded in the brief glow of rosy optimism that followed the ending of the Second World War, and in the belief that there are ways in which nation-states may benefit from each other's enlightenment, UNESCO has nevertheless been a bitter disappointment to its well-wishers. The few achievements it can fairly boast of (it has a nasty habit of boasting of others' achievements as if they were also its own) would probably have been brought about by other agencies if UNESCO had never been founded. Now, and for the past five years, UNESCO has become a divisive influence in international relations. Many of its recent initiatives seem almost to have been calculated to cause trouble. Thus the programme to launch a "new information order" would have given governments, and malevolent governments in particular, an open licence to control the flow of information about themselves. In its smaller works, UNESCO shares with the Greater London Council the distinction of being one of the most generous sources of support for causes that are saved from wrong-headedness only by their impracticability. It is also extravagantly run and badly administered.

These are some of the reasons why the United States, once responsible for 30 per cent of UNESCO's budget, quit with effect from the end of 1984. A year ago, Britain took the first step in that direction, saying (in a letter to the director-general, Mr Amadou-Mahtar M'Bow) that it would decide after the meeting of UNESCO's governing body at Sofia this month whether to pull out as well. A British decision to withdraw would probably be followed by a few others, but not many. By all accounts, the British government has not yet made up its mind. Sofia will hardly help. Like previous meetings of this kind, there has been the usual crop of arguments about the validity of Israeli and other credentials to attend the meeting. Decisions to cut spending to match income (now reduced) have been resolved but not yet implemented. UNESCO's programme has been trimmed here and there, but there is still no sign that the organization is any nearer getting to grips with the underlying difficulty: how to tackle sensibly the vast array of problems specified by its charter. Those inclined to walk out last year cannot convincingly pretend that the reforms they were demanding then have since become realities.

Changes

What has changed, partly as a consequence of the withdrawal of the United States, is the political climate in which UNESCO exists. The organization's natural supporters have closed ranks behind Mr M'Bow. The poorest countries and those part-way through the process of development, India for example, say that they are being robbed of an important channel of technical assistance. Governments unconvinced of the case for staying in UNESCO find their scepticism smothered by the fear that UNESCO will become a Soviet-led cabal legitimized as a UN agency and nursing a grievance against the industrialized West. So governments still represented at Sofia tend to look on the bright side of what is happening, mistaking talk of reform for the reality. The result, so far, is that the Sofia meeting has been unexpectedly harmonious, the usual skirmishing notwithstanding.

The hidden danger in this appearance of amity, for UNESCO's critics, is that compliance now will be taken as acquiescence in UNESCO's traditional pattern of activities. And the plain truth is that none of the clamour for reform in the past few years has been radical enough to conjure up an organization that would be capable of fulfilling the terms of the original charter, let alone of giving the remaining members a sense that their subscriptions are being wisely spent.

Criticisms

Most of the critics' complaints against UNESCO are directed against the symptoms of the organization's underlying trouble, not against its causes. It would be wise, even at this late stage, if both UNESCO's critics and friends could make a more objective appraisal of the organization they have inherited from four decades of thoughtless neglect by member governments.

The scale of the organization is important. UNESCO's budget, with extra contributions for special projects but without the US contribution, amounts to less than \$200 million a year, which means that UNESCO is financially smaller than many major universities and smaller even than the substantial private foundations in the United States. Yet the charter requires that the organization should take an interest in the whole of scholarship, culture and education, while its tradition, accreted accidentally during the past four decades, is to attempt to function as if it were a grant-making agency. Of necessity, of course, UNESCO seeks to spend a little money so as to induce others to spend much larger amounts (and then makes a virtue of necessity by describing itself as a catalyst). One inevitable consequence is that most of UNESCO's schemes are poorly supported, cumbersome to manage and indecisively executed. In the competition for good ideas, it gets the leavings. Another consequence paradoxical for a would-be catalyst is that UNESCO is pathetically eager to continue supporting its successes, precisely those projects from which it should be quick to walk away. So UNESCO should set out more deliberately to be an advocate of good causes which others must support. In present circumstances, there is not the slightest possibility that UNESCO would ever have sufficient funds to match the foundations that operate internationally, let alone its own ambitions.

But how could an organization without money to jingle in its pockets ever hope to accomplish anything? This stock response, from UNESCO management, is a measure of the gap that has opened between the ideals of the charter and present practice. The simple answer is that there is plenty that could be done, by an intelligently organized UNESCO, that would require neither the present huge staff of several thousands nor the token grants with which the organization attempts, usually in vain, to kindle fires here and there. For instance? If high-energy physics is really getting to the point at which no single sponsor can afford the next, or the next-but-one, accelerator, may there not be a role for an international beater of heads together? With the less developed countries crying out for technology transfer without knowing what the phrase means, or what the consequences would be, should not UNESCO or some other body step in to help? (The most successful transfer of technology so far, that of plant breeding, has been sponsored by the World Bank, with UNESCO standing dumbly on the sidelines.) The hard prob-



lems of securing the independence of academic institutions which, perforce, must be supported with public funds, deserve a more thorough international hearing than they have had. Even the handling of news on an international basis is a fit subject for discussion, contentious though it would be; UNESCO's fault so far is that it has sought to market a solution for a problem it has not bothered to define.

But changes along these lines would damage the few good things that UNESCO does at present; that is what the cautious will protest. Certainly UNESCO without an operating budget would be unable to provide the annual subvention that maintains the Paris headquarters of the International Council of Scientific Unions, the secretariat of the International Commission on Oceanography and the general drum-beating on behalf of the Man and Biosphere programme. But these are precisely those parts of UNESCO's present programme whose value is generally accepted and that will find support from their beneficiaries even if UNESCO vanishes without trace. UNESCO even as it is can take pride in having backed these good causes but can confidently leave their future in other hands, saving its energy for the more difficult tasks that remain untackled. UNESCO needs to become what it pretends it is already, an intellectual catalyst.

Reform, along lines such as these, would mean a UNESCO very different from what now exists. Ironically, it would also be an organization that spent an even greater proportion of its resources on itself, which only goes to show the United States and Britain are mistaken in complaining that one of UNESCO's present sins is that it spends too much on itself. That complaint starts from the premise that UNESCO is, or should be, yet another technical assistance agency of the United Nations. But there are too many of those already. What UNESCO should be is what it was designed to be, a cross between a kind of international academy of all the sciences and humanities and a body capable of knowing (and saying) what public policies would advance that cause. Present attempts to make UNESCO more efficient will push it in an opposite direction.

So the reformers should ask themselves afresh what tasks are implied by UNESCO's charter, what functions can be accomplished with a budget of the present size (or even smaller) and what machinery might serve the purpose. Their conclusion, no doubt, would be that a smaller group of people (few of whom would be drawn from the present bureaucracy) might, by canalizing the energies of far-sighted people in other jobs, perform an international public service on behalf of scholarship and culture. Having no money to spend (except, perhaps, on people's travelling expenses) would entail the beneficial conclusion that wrong-headed schemes such as the new information order could not be foisted on unwilling subscribers except with their consent. Britain and like-minded critics of the present regime, having made a fuss without quite knowing why, should not now pretend that the reforms on offer at Sofia are a sufficient response. Instead, they should demand that UNESCO must become what it was meant to be and then declare their willingness to stay to bring that end about. Pulling out is the easy option, which the member governments have made seem sensible by their indifference to what UNESCO has been up to all these years. The end of Mr M'Bow's present term of office two years from now should be a chance to put things right. □

Summit talking

Preparations for next month's summit meetings are getting out of hand.

THE blind date that has been arranged between President Ronald Reagan and Mr Mikhail Gorbachev at Geneva next month will be a strange affair. The objective is said to be that these two powerful politicians should find a way of cutting through the difficulties that at present divide them, especially on strategic arms control. But the two people have not previously met each other in the flesh, nor exchanged a single word of

conversation, civil or otherwise. In circumstances like these, the ideal would be that the blind date should be as little hedged about with restrictions as may be possible. It might even be better to agree in advance that no decisions will be made at this first meeting, but that there will be another in a few months or so. So why are the two men and their assistants apparently determined to have their conversation in advance, in public? And on specifying what they will or will not agree at Geneva in such detail that there will be precious little to talk about when they meet?

The most serious issues to have emerged from the torrent of public speaking in the past few weeks are three — the US plan to develop a defence against missiles (the Strategic Defense Initiative or SDI), the sanctity of the Anti-Ballistic Missile (ABM) Treaty (1972) and the development of anti-satellite weapons (one of which was tested for the second time by the United States last month). If some kind of understanding can be reached on these questions, there seems no reason why the patient negotiating teams also at Geneva should not be able to reach an agreement on something like the Soviet plan for a fifty per cent reduction of strategic warheads (but that will take time). Unfortunately the two sides are busily painting themselves into different corners by insisting on what they will not concede.

SDI is the most serious stumbling block, if only because so much has been said about it, but is clearly linked with the ABM treaty. The United States is right to insist that a ban on research is unverifiable and therefore unacceptable, which Mr Gorbachev appears to accept. But the United States is wrong to say (as the US Department of Defense said last week) that testing components of SDI would be compatible with the treaty provided that they were not complete weapons systems; one objective of the treaty was to prevent just the kind of development now in prospect. And it does not affect the integrity of the treaty to point to the recent development of anti-missile defences in the Soviet Union, as the US Secretaries of State and of Defense did in their detailed account of Soviet hardware put out last week; either these developments are compatible with the treaty (which allows the missile defence of Moscow) or they are violations, and they should be dismantled.

The way out of this fix is that the ABM treaty should be modified so as to allow the provision of better early-warning systems (see *Nature* 3 October, p.371) but otherwise reaffirmed. The problem of anti-satellite weapons should be dealt with in the same way. In 1972, when the ABM treaty was signed, the strategic importance of military remote-sensing satellites was not as evident as it has become. The case for making sure that one side cannot knock out the other's warning and communications systems is strong. So the ABM treaty should be amended to include an interdiction of the deployment of anti-satellite weapons. Testing should also be banned. But, the US State Department and the Pentagon say, the Soviet Union has already ground-based launching systems ready to be used against US satellites. The information is so specific as to be plausible, but the solution is simple: demand that the hardware in place should be destroyed and not rebuilt.

Steps like these seem reasonable enough, but it is a lot to ask that the summiteers should take them when the general climate of the relationship between their two countries is at such a low ebb. And while an arms control agreement might reasonably be expected to contribute to better relationships, it is dangerous to hazard agreement in that vital field on the chance that next month's blind date will work out. The two concerned had better plan to talk about some less contentious issues as well. □

Biological manuscripts

Contributors are reminded that, with the transfer of the Biological Sciences Editor to the Washington office, they should in future send **four copies of all manuscripts** offered for publication either (as at present) to London or to Washington.

Transputers

Inmos puts its head in the lion's mouth

Tokyo

SELLING microchips to Japan is not a task for the faint of heart. But Inmos, the United Kingdom's only microchip company and a pygmy compared with the eight giant Japanese electronic corporations, is trying to do just that. Last week the revolutionary product on which they pin their hopes — the transputer — was launched in Tokyo, as well as in the United States and the United Kingdom.

To avoid being crushed underfoot, Inmos has aimed at a product that does not compete directly with anyone else's. The transputer is claimed to be the first "computer on a chip"; on less than a centimetre square of silicon are a programmable processor, a 2K random access memory and a special set of four two-way communication channels. These features make the transputer not only a powerful microprocessor in its own right but also give it the capability to be linked up to many

other transputers — tens, hundreds or perhaps even thousands. Thus the transputer may provide a building block for computers of the future that work in parallel rather than sequentially.

There are good reasons for building parallel computers, although enormous conceptual difficulties remain in building a general-purpose parallel machine (see below). In principle, each time the number of processors is doubled, the processing time is halved — and the sky is the limit. Of course, it is not so easy to divide up problems among processors to get linear increases in speed. But Inmos does have a few convincing demonstrations of what can be done, given the right problem. One example is that of generating a two-dimensional picture, complete with shadows and highlights, of several three-dimensional coloured objects illuminated by lights. The problem is not easy because each object will contain reflections of all

the other objects as well as the lights. It can be solved by tracing the path of each ray of light; and as the rays are unaffected by one another it is possible to divide the work among processors — the more transputers Inmos puts onto the problem the faster it is solved. Similar logic has been applied in building a machine to encode and match fingerprints for Scotland Yard.

The possibilities for inventive designs incorporating transputers are endless. And it is those who are looking for new parallel solutions to tricky problems who are likely to be among the first customers for the transputer. Indeed, such people are already banging on Inmos's door. But for financial success Inmos has to look beyond these customers to the conventional microprocessor market and that will be a harder nut to crack. Used as a single 32-bit microprocessor (the kind of chip that forms the heart of personal computers, for example), Inmos claims that the transputer is still very considerably faster and more efficient than microprocessors produced by Intel or Motorola, the two US companies whose designs dominate world markets. And it is very easy to add more transputers when more facilities are required. But Intel and Motorola are already well established.

Beyond the conventional microprocessor market may come a third wave of buyers as the possibilities offered by parallel processing become more widely appreciated by conventional programmers. Indeed, it is even possible that OCCAM, the transputer's special programming language, will become a world standard for parallel applications.

But Inmos has first to establish itself in the microprocessor market. The next two years should show whether the transputer will be a world-beater or whether, like the rear-engined jet and the hovercraft, it will turn out to be one of those British ideas that are too far ahead of their time.

Alun Anderson

Why should computers go parallel?

Tokyo

A CONVENTIONAL computer operates step-by-step by fetching data from a central memory store, operating in it and returning the result to the store. To crack huge problems, like those of modelling the behaviour of the atmosphere or nuclear reactions, or to produce the computational power needed for "intelligent" applications, computers are being built with central processors that can perform one operation after another at ever higher speeds. But the limits to this approach are already on the horizon.

The day is coming when computers will perform operations so quickly that even at the speed of light there will not be time to fetch data from memory stored more than a few tens of centimetres away. So computers will have to become more and more compact, but as they do it becomes impossible to disperse the heat they produce. That is one reason why researchers are trying to find ways to use many processors working in parallel; another is that the staggering engineering problems that have to be overcome to build the next generation of supercomputers will make them prohibitively expensive.

Solving a problem in parallel is much like tackling a real-life organizational problem, say that of running a journal. Different parts of the total task can be assigned to different editors, all working in parallel. But their efforts have to be synchronized and information passed back and forth between them (and outside con-

tributors) in order that the final goal, production of a complete journal, can be achieved. The difficulties of dividing up a problem, ensuring that different parts are done at the right time, passing the right information back and forth between processors, and making sure no processors are sitting idle, have to be solved to build an efficient parallel machine. Some types of problem will require individual processors to be powerful and fairly independent, as in image processing where the same operations can be performed simultaneously on every part of the image. Others will require enormous information exchange between different processors and thus enormous interconnectivity.

The transputer can provide the basic building block for the construction of such parallel machines, as its on-chip memory and special communication channels enable it to talk to other transputers while performing operations. Even more important is that the transputer has been built around a special language, OCCAM, developed at the University of Oxford for parallel applications. OCCAM commands make it easy to specify what set of processes are to be performed in parallel and to define communication channels which come into use when receiver and sender are ready to pass information. Indeed, OCCAM is the first practical language to provide a simple means of controlling concurrent operations; it already has 5,000 users worldwide including an enthusiastic band in Japan.

Alun Anderson

Soviet hostages dead

Two Soviet geologists who were kidnapped by rebel forces in Mozambique in August 1983 are dead, the Mozambican foreign ministry has announced. The geologists, Yuri Gavrilov and Viktor Estamin, were in Mozambique under a scientific and economic cooperation agreement, as advisers to the Mozambican mining industry in Morrua, Zambezi province. During a rebel raid on 21 August 1983, 24 Soviet geologists were captured; most, however, were subsequently released as a result of military action by government forces and diplomatic negotiations. Gavrilov and Estamin remained in rebel hands.

This brings the total of Soviet victims of the Morrua raid to six; two were killed in the raid itself, while two others were known to have died of hardship during their captivity.

Vera Rich

Niels Bohr

Centenary revives old questions

Copenhagen

THE eight hundred or so who gathered at the Niels Bohr Institute last week to celebrate the centenary of Bohr's birth were united in their awe and admiration of the quantum theory, which they agreed has been colossally successful at every level that it touches. Yet the gathering also vividly showed how the old questions, mostly first asked by Bohr himself, continue to haunt those who think about the quantum mechanical description of the world.

Anthony Leggett of the University of Illinois put the dilemma clearly. "By day, you can see me sitting at my desk solving Schrödinger equations and calculating cross-sections just like any other physicist. But occasionally, at night . . . I question whether quantum mechanics is the complete and ultimate truth about the Universe . . . Worse, I am inclined to believe that at some point between the atom and the human brain, the superposition principle must break down."

But even those who marvel at the theory Bohr built do not suggest that the limitations of quantum theory have been fully explored. Does quantum mechanics, successful on the scale of one or a few atoms, function when there are large numbers of them? Schrodinger's paradox of the cat in a box reappeared last week, but in a refined form. The cat may be in one of two states, alive or dead, and in the absence of information one way or the other must be assumed to be in a superposition of the two. But when the box is opened and the cat observed, only one state *i* is possible.

The underlying question is whether the essence of the quantum mechanical description, the superposition of states, does carry through from the microscopic to the macroscopic. Leggett last week described a superconducting ring containing a Josephson link that may be used to test the issue. Current can flow in one direction or the other, but can it be said to be flowing in both directions at the same time? Leggett's subversive hope is that "common sense" will prevail, in which case quantum mechanics will have to be adjusted for large numbers. But most last week held that common sense would fail and quantum mechanics triumph: the experiments of Aspect and his colleagues, the argument goes, have already shown that, at the microscopic level, one cannot talk of a "real" world independent of the observer without breaking the barrier to the velocity of light.

That would have delighted Bohr, but he would have been surprised at the degree to which arguments involving symmetry, and symmetry-breaking, have become features of physical explanations. Philip Anderson (Princeton) applied the notion

to the nature of the measurement process, while Robert Schrieffer (Santa Barbara) showed how symmetry-breaking leads to fractional quantum numbers in systems as different as electrically conducting polyacetylene, two-dimensional heterojunction semiconductors and the Bose-Dirac fields of relativistic field theory.

Symmetry, or symmetry-breaking, comes into its own in particle physics, of which Sheldon Glashow (Harvard) gave a characteristically racy account. The quarks and leptons which seem to be the fundamental particles of our world, and the characteristic forces between them, are the consequences of a broken symmetry obtaining at an earlier stage of the Universe, when characteristic energies were greater than now.

The cloud hanging over particle physics, Glashow said, is the very success of the "standard model" built up in this way. There are no contradictions, no loose ends and no hint of structure below the quarks and the leptons now recognized. And recently there has also been a dearth of surprise. From the 1930s to the 1970s, each decade saw the discovery of unexpected particles, but this decade (so far) has seen only the discovery

of the W and Z particles — a "fantastic achievement, but not a surprise".

Indeed, in Glashow's view, the characteristic of this decade is the list of missing surprises — magnetic monopoles, neutrino oscillations, free quarks, proton decay, unpredicted particles and the like. Maybe, Glashow teased, the subject has become like medieval scholarship, with experimenters endlessly refining (like the alchemists) ancient techniques in their search for the exotic, the theorists (or theologians) in a realm based on faith where all things of interest are inaccessible (with particle accelerators what they are) and where truth depends on elegance and completeness.

Steven Weinberg (Austin, Texas) has no qualms about his theoretical devotions, now to the theory of superstrings. The theory, Weinberg said, is the product of a post-Bohr dialectic between quantum field theory on the one hand and scattering-matrix theory on the other. Weinberg decided last January "to drop everything" for the theory of superstrings, which avoids in a natural way the mathematical difficulties of other approaches to the unification of the forces of nature and "which has that flavour of uniqueness that we look for". Unashamed, he said that there is not yet the slightest evidence for this elegant theory.

Philip Campbell

US-China nuclear pact

Foes in Congress speak out

Washington

PRESIDENT Reagan's nuclear technology transfer agreement with China ran into more problems last week, with the admission by the Nuclear Regulatory Commission (NRC) that the nature of China's assurances on the implementation of nuclear policies "could lead to future misunderstandings". NRC is required by its Nuclear Anti-Proliferation Act (1978) to comment on proposed agreements. NRC would have preferred the agreement to contain a "clear statement of US consent rights for the subsequent reprocessing of enrichment of US-supplied nuclear fuel", it revealed in a letter to Senator William Proxmire (Democrat, Wisconsin), one of the main critics of the agreement. NRC last week would not discuss its concern in detail or comment on allegations that China has helped Pakistan to build nuclear weapons. But NRC provided testimony to Congress in a closed session last week.

Congress is examining the issues raised by the cooperation pact and has until the end of next month to act if it wants to try to alter any of its provisions. Testifying before the Senate Foreign Relations Committee last week, Professor John Cooper of Rhodes College, Tennessee, described the "periodic and extreme" shifts in China's foreign policy during the past 35 years and said that China's record on ex-

port policy is "not good".

Paul Leventhal, president of the Nuclear Control Institute, told the committee that the agreement is "seriously flawed" in its "legitimizing" of plutonium, neglect of safeguards and potential for disputes. Senator John Glenn (Democrat, Ohio) has just introduced legislation embodying some of these worries. His bill seeks to tighten safeguards and demands documentation of China's non-proliferation policy, conditions that would make the agreement "salvageable", he says.

Edward Luck, president of the US United Nations Association, told the committee that trying to force China to make further assurances would be "counterproductive" given the efforts already made to meet US concern about non-proliferation. Energy Secretary John Herrington and ambassador Richard Kennedy, negotiator of the agreement, told the committee that both countries would gain "significant benefits" on ratification of the agreement.

It is difficult to predict whether Congress will try to alter parts of the agreement, the first of its kind between the United States and a single nuclear weapons state. The opposition to the pact is thought to be a "significant minority", unlikely to put President Reagan in the embarrassing position of having to renegotiate with China.

Maxine Clarke

Arianespace

Rocket company to sell insurance?

ARIANESPACE, the company responsible for commercial exploitation of the European space launcher Ariane, is working out a scheme to offer its customers partial insurance against the risk of failure. This development is a response to the rising cost of satellite insurance on the open market, which may now be as much as 30 per cent of the capital cost of a satellite.

Arianespace plans to offer insurance against the risk that satellites will be lost during the launch period. Satellite operators will have to arrange separate insurance against the risk of failure of the payload, which includes not only the satellite but the rocket motor by means of which the satellite is transferred into its final orbit from the apogee of the highly elliptical injection orbit.

Under the insurance plans, the company would charge users a standard premium based on the risk of failure spread over as many as 15 launches in a three-year period. Arianespace would protect itself in the commercial reinsurance market. Before the failure of last month's launch of Ariane, insurance premiums were running at 18–20 per cent of satellite costs, of which 10–12 per cent is estimated to have covered launch failure and 7–8 per cent the risk of satellite failure. Ariane users will have to cover the second risk independently. Arianespace says that it is still negotiating with insurers and so does not yet know what premiums it will charge.

The scheme may nevertheless be ready in time for the planned launch of the French Earth observation satellite SPOT 1, perhaps two months from now, but Arianespace is more concerned with the string of commercial users that have booked into succeeding flights. It thinks that attempts to charge them insurance premiums up to 30 per cent of capital costs are "completely stupid", but is also clearly alarmed about the effect on the long-term demand for launching services.

A successful launch insurance scheme with modest premiums, on the other hand, should give Ariane an advantage in competition with the US shuttle. In France, there is much resentment at reports that charges for shuttle flights are often only 20 per cent of real costs. Ariane's advantage over the shuttle is that much less rocket power is needed to transfer a satellite to geostationary orbit from the apogee of the injection orbit than from the low-altitude circular orbit of the shuttle, with the consequence that the risks of failure must be less.

Meanwhile, the failure of Ariane's fifteenth launch on 13 September has been explained by an independent commission of inquiry. Arianespace technical director, Dr Klaus Iserland, said last week that the chief problem appears to have been a leak in a valve between the liquid-hydrogen

tank of Ariane's third stage and the combustion chamber; the leak has been traced to an extra flange, designed to make the liquid-hydrogen valve fit more tightly, which was added in the eighth Ariane flight. The valve's differential expansion allows it to leak at intermediate temperatures, and in particular when it is being cooled by the first flow of liquid hydrogen.

Arianespace has now asked the manufacturers to remove the extra flange. The "earliest possible hypothetical date" for the next launch, according to Iserland, is mid-December. If Arianespace can hold to that, SPOT 1 will be only one month late.

Robert Walgate

Skull-trade ban

New Delhi

INDIA, which prohibited export of rhesus monkeys for medical research, has now banned the export of skulls and skeletons for medical colleges abroad. The ban follows shocking reports in the Indian press about children being kidnapped and killed for their skulls.

The article in the *Pataliputra Times* (owned by Dr Jagannath Misra, former chief minister of Bihar) claimed that traders in skulls and skeletons in Bihar receive children from gangs of kidnappers who are paid £35 to £70 per child. It said that the children are handed over to head-choppers at Bangshat on the bank of the Ganges who charge £2 for chopping a head. The headless bodies are thrown into the Ganges while the heads are stripped of skin, boiled and bleached for export. The report claimed that 1,500 skulls processed this way are sent to Calcutta each month by rail for export to 23 countries. The article claimed that 5,000 to 7,000 children are reported missing every year in the state.

These revelations created a furore in the Bihar state legislative assembly. The police arrested a man suspected of being a kingpin of the trade, but he was released on bail. The state government however said that skulls found in three boxes meant for export probably belonged to dead bodies floating in the Ganges. It is a fact that poor Hindus who have no money to buy firewood for cremating the dead simply throw the bodies in the river.

The skull trade dates back to 1936. A ban on skeleton export imposed in 1976 was lifted in 1977 when there was a change in the central government. Since then India has reportedly been meeting 80 per cent of the world's requirement of skulls and skeletons with Bihar alone contributing 60 per cent. Thirteen companies in Calcutta had licences for export as of August this year, when the government imposed a fresh ban.

K. S. Jayaraman

US space science

Two paths to leadership?

Washington

SHOULD US space science be concentrated on a few large projects, or instead be spread more widely? Congress heard both views last week, at a committee meeting looking ahead to financial year 1987, which is still a full year off. The National Aeronautics and Space Administration (NASA), according to Noel Hinners, director of the Goddard Space Flight Center, has as a top priority the creation of "a set of long-life space observatories complemented by ground-based facilities". The space shuttle makes this plan feasible by permitting the repair, maintenance and improvement of instrumentation satellites. The delayed Hubble space telescope, now due to be launched next year, is the first of these planned observatories, and will be followed by the gamma-ray observatory in 1987. The solar optical telescope and the space infrared telescope facility, programmes originally recommended by US astronomers in 1972, have been "seriously delayed", but are still planned for the 1990s if Congress will pay for them.

Hinners also wants Congress to allocate funds for the advanced X-ray astrophysics facility programme, planned for a 1987 start but likely to be postponed when next year's appropriation is decided in mid-1986. Since the Einstein observatory went out of action, European and Japanese groups have taken the lead in this field, according to Jeffrey Hoffman of NASA. He says that Congress must approve the programme soon if the United States is to regain its supremacy in X-ray astronomy. Another project waiting on Congress is the large deployable far-infrared detector, which NASA hopes to launch towards the end of the 1990s. James Van Allen of the University of Iowa is on the other side of the argument. Van Allen was one of the first users of the US vanguard satellites in the 1950s, when he discovered the clouds of charged particles trapped in the geomagnetic field. He wants NASA to spend less on major programmes and to increase its budget for smaller, flexible and potentially more productive projects involving more laboratories.

There was no shortage of proposals for such programmes last week. One was the international solar terrestrial physics programme and another an Earth observing system to coordinate data collection on climate, geography and chemistry. But NASA is assured of only one per cent per year budgetary growth, which means, says Van Allen, that space science and applications are likely to experience "hard times . . . for many more years as planned expenditures for the space station increase".

Maxine Clarke

AIDS

US law delays drug testing

Washington

PLANS by the National Institute for Allergy and Infectious Diseases (NIAID) to speed clinical trials of potential treatments for some of the consequences of acquired immune deficiency syndrome (AIDS) have been thwarted by a legal technicality. Nine clinical research centres around the United States that had been awarded "master agreements" to conduct phase II clinical trials of antiviral drugs and therapies for opportunistic infections to which AIDS patients are prone have been told their agreements will not be honoured because of new government contracts. Future trials will be handled with standard

procedures that will lead to routine delays of about two months, according to Carl Fretts, director of NIAID's division of contracts and grants.

So far, clinical trials of potential AIDS therapies are still in the phase I stage, aimed at establishing safety and using small numbers of patients. Under the master agreement scheme, testing centres able to demonstrate adequate facilities and expertise for controlled phase II trials were certified in advance by NIAID. When trials then became necessary, the best-qualified centres could be quickly selected and protocols drawn up with a minimum of delay. The scheme freed NIAID from the requirement to advertise each trial contract for 30 days before considering proposals; it would also escape having to consider proposals from unqualified institutions. It was "a perfect mechanism", according to Harry Haverkos, AIDS project officer for NIAID. Similar arrangements have been used in the past for trials of anti-cancer drugs.

To qualify for a masters agreement, institutions had to produce "an enormous amount of information", according to one disgruntled researcher. Centres were required to show that they had sufficient numbers of patients, adequate laboratory expertise and facilities for handling large amounts of data. The nine successful centres were selected last May (from a considerably larger number of applicants).

Only one contract for an actual trial had been awarded when NIAID realized that the agreements are apparently illegal under the Competition Act, which came into effect in April. The scheme is now "in limbo", according to Haverkos. The single contract awarded under the scheme was to Robert Holzman of New York University Medical Center for a controlled trial of the effectiveness of trimethoprim-sulfamethoxazole and Fansidar in preventing recurrences of *Pneumocystis* infections. Holzman is now waiting for delivery of the drugs to start the trial.

George Galasso, coordinator of AIDS clinical trials for the National Institutes of Health, "does not believe" the legal foul-up has delayed getting trials under way, since many institutions are willing to proceed with trials even without a formal source of funds. Others, including some researchers, disagree. Haverkos would not exclude the possibility that trials have been delayed, although "we couldn't have got more than four or five under way" by now even if all had gone according to plan.

According to Carl Fretts, the Competition in Contracts Act was passed by Congress primarily to curtail abuses by defence contractors and was never intended to apply specifically to clinical trials. Through the Department of Health and Human Services, NIAID will bring the

matter up shortly with the Civilian Agencies Acquisition Council, which wrote the implementing regulations, and seek to have the regulations altered so that masters agreements are permitted. Fretts points out that such agreements do not stifle competition because research centres have to reapply once a year. In the meantime, NIAID will by next month be awarding standard contracts for the trials, and special dispensations to speed future rounds of the contract procedure are being sought.

Tim Beardsley

Plant protection

US patent rights march on

Washington

THE US Board of Patent Appeals and Interferences has ruled, in what is hailed as a "landmark decision", that seeds, plants and plant tissue cultures can be protected under the 1952 Patent Act. The decision reverses existing government policy and is expected greatly to increase incentives for research in plant biotechnology.

The board's ruling was a response to an appeal by Molecular Genetics Inc. which has modified maize plants to produce extra tryptophan, an amino acid that has often to be added as a supplement to animal feed. Tryptophan-enriched maize used as animal feed would avoid the need for supplements.

Before the recent ruling, only limited patent protection could be obtained for plants. The 1930 Plant Patent Act covered asexually reproduced plant varieties, and the 1970 Plant Variety Protection Act covered single novel varieties of sexually reproducible plants, but neither has been considered adequate by the plant biotechnology industry. The hybrid offspring of a patented plant variety and a standard strain would not, for example, be protectable under the 1970 act.

Because of the new ruling, plant biotechnology will receive "the same generic scope of protection as other inventions", according to Leslie Misrock, patent attorney for Molecular Genetics. Companies that patent novel seeds, plants or tissue culture will be able to prevent competitors from making, using or selling the protected item — which includes using it for breeding. The effect for Molecular Genetics will be that, once its patent is issued, not only its original tryptophan-enriched maize seed but all subsequent varieties bred from it will be protected.

The company intends to produce a range of tryptophan-enriched maize seed with different varieties for different climatic conditions. Misrock said that the implications for the seed industry of the new decision are "enormous". It will, he said, transform it "from a speciality industry to a commodity industry".

Tim Beardsley

Beckman bucks

Washington

THE University of Illinois is to receive \$40 million, the largest gift ever by an individual to a public university, from Arnold Beckman, founder of Beckman Instruments Inc. The money will be used to establish a multidisciplinary research institute consisting of two centres, one focusing on materials and computing science and the other on biology, behaviour and cognition.

Research at each centre will begin with problems at the atomic and molecular level and proceed through systems of increasing complexity. The ultimate aim is to understand "intelligence", says Theodore Brown, chancellor for research at the university. Emphasis will be placed on a comparison of complex integrated electronics and artificial intelligence systems with biological systems.

The university sees the centre as the largest-scale attempt yet to bridge the gap between the physical and biological sciences in one institution. A grant of \$10 million by the state of Illinois will make possible a 1986 start on construction at the site, which should be completed by 1988. The state has also guaranteed the institute \$2 million a year in perpetuity for running costs and the launch of research programmes. The bulk of the financial support for running the institute is expected to come from outside sources. A director should be appointed within six to nine months. Beckman, now 85, began the company of which he remains chairman in 1935, when a member of the faculty of the California Institute of Technology. His first product was a pH meter, but by the time the company was merged with Smith Kline Corporation in 1982, Beckman was selling more than \$1,000 million worth of instruments each year. There seems to have been some genteel competition for Mr Beckman's generosity between Caltech and Illinois, where Beckman took his first degree (in physical chemistry) in 1923.

Maxine Clarke

Nobel Prizes

Cell cholesterol wins the day

WHEREAS the division of the spoils is often controversial when it comes to Nobel Prizes, there will be no argument about the sharing of this year's Physiology and Medicine award between Michael S. Brown and Joseph L. Goldstein, for their collaboration has been remarkable by any standards. Since meeting as medical students in the 1960s, they have been apart only for a brief spell while Brown boned up on biochemistry and Goldstein on genetics, before setting up, in 1972, a joint programme on cholesterol metabolism at the University of Texas Health Services Center in Dallas where they continue to collaborate as equals. Of the "discoveries concerning regulation of cholesterol metabolism" for which the prize has been awarded, pride of place must go to Brown and Goldstein's relentless study of the cell-surface receptor for low-density lipoprotein (LDL). LDL is the major transporter of blood cholesterol and the LDL receptor is the key to the transfer of cholesterol from the blood to the interior of fibroblast and liver cells.

Brown and Goldstein have hounded the receptor with a combination of biochemistry, genetics, microscopy and, most recently, molecular genetics. As a result, the LDL receptor is more thoroughly understood than any other receptor and its study has been highly influential in promoting the general investigation of receptor-mediated endocytosis — the process by which many extracellular molecules are delivered to the interior of cells. In essence, Brown and Goldstein have demonstrated that complexes of LDL and receptor concentrate in "pits" formed in the cell-surface membrane and thence in vesicles that are pinched off from the pits. After fusion of vesicles in the cell's interior, the receptor and LDL part company. The receptor is recycled to the cell surface whereas the LDL is degraded in the cell, freeing the cholesterol from the protein constituents of LDL, so that it can be utilized in the cell.

Without a doubt these studies have benefited enormously from the existence of rare hereditary defects in the receptor, which result in the condition known as familial hypercholesterolaemia. Through molecular genetics, Brown and Goldstein have been able to discover exactly what is at fault with the receptor's gene in some patients, and therefore precisely why their receptors are defunct (*Science* **227**, 140; 1985, and *Cell* **41**, 735; 1985). For patients with a pair of defective genes, the result is excessively high blood cholesterol and LDL, with the development of coronary atherosclerosis usually before the age of twenty.

Not content with studying the receptor-mediated uptake of cholesterol into cells, Brown and Goldstein have recently col-

laborated with Paul Berg, a 1980 Nobel laureate, in cloning the gene for the key enzyme of cholesterol synthesis in tissue (*Nature* **308**, 613; 1984). The enzyme, known chiefly as HMG CoA reductase, is of particular importance because its production is subject to negative feedback regulation by cholesterol. If the mechanism of that feedback can be understood in terms of the gene, it will be possible to explore the possibility that gene defects

US diet

Back to the stew-pot

Washington

A PUBLIC row has erupted in the United States because the National Research Council has rejected the conclusions of a dietary study it commissioned five years ago to make new Recommended Dietary Allowances (RDAs) for nutrients and energy. The chairman of the study committee, Henry Kamin of Duke University, questions the competence of the research council's anonymous reviewers and accuses them of being "scared" of the policy implications of his report. Among other things, he would reduce RDAs for vitamins A and C, as the study wanted. Kamin blames the decision not to publish the committee's report on the "vulgarization of nutrition" in the United States.

Kamin's study was to have been the 10th edition of the research council's *Recommended Dietary Allowances*. Since they were first issued in 1941 as a guide for planning national food supplies, RDAs have expanded both in number and variety of applications, and are now central to many federal food assistance schemes. Kamin's committee was asked to derive RDAs "adequate to meet the unknown nutritional needs of practically all healthy persons".

The figures Kamin eventually arrived at differ — though usually by small amounts — from existing RDAs, but were "both rigorous and original", according to Kamin. Reviewers appointed by the Research Council believed, however, that modifications to RDAs should be made only in the light of "compelling new evidence" — which, they said, Kamin failed to provide.

After six months of revisions and re-revisions, reviewers and study authors were still unable to agree on what should be the proper RDAs for vitamins A and C, with some unresolved questions over the RDA for calcium (which, in contrast to those for the vitamins, the study authors wanted to increase). Frank Press, chairman of the research council, then stepped in to call a halt, and will now establish a new committee with a revised brief to take up the question raised.

are a factor in some cases of hypercholesterolaemia.

While it is still too early to be certain of the relevance of much of Brown and Goldstein's work to the prevention and treatment of atherosclerosis, their work has been of great fundamental importance and highly influential. So far all attempts to lure both of them from their opulent Dallas surroundings to more distinguished institutions have failed. Nobel prizes seem unlikely to make any difference nor to split such a close working partnership that no paper is published by either partner without the other.

Peter Newmark

One of the key differences between Kamin and the reviewers is whether the evidence needed to justify changing an existing RDA should be stronger than that needed to establish a completely new value. Some of the changes recommended by Kamin's committee were based on reinterpretations of the studies on which the existing RDAs are based; not all of these were accepted by the reviewers. But, according to Kurt Isselbacher, chairman of the research council's Food and Nutrition Board, Kamin's committee was "reluctant to accept criticisms" and displayed "tremendous inflexibility". Given the differences of opinion, the research council then had to decide whether it should issue new recommendations that were "scientifically no better than the old ones".

Another area of dispute was the weight that should be attached to the public impact of the recommendations. Despite Kamin's accusation that he was effectively asked "to steer the science to fit the policy implications", Isselbacher maintains that policy worries "were not a concern that was felt by most". Nevertheless, policy implications did provoke the question whether the scope of the study was adequate to formulate new RDAs, given new evidence about the relation between diet and some chronic diseases. For example, Kamin, concerned only with protection against nutritional deficiency, proposed a lowering of the RDA for vitamin A, but another report from the research council says that vitamin A might help to prevent cancer. Some reviewers were concerned about the effect on the public of such apparently contradictory recommendations.

For these and similar reasons, according to Frank Press, the next edition of the RDAs will include "a more encompassing analysis of data pertaining to nutrients and health". The new study group is to be chosen in consultation with the National Institutes of Health. In the meantime, writes Press, the public "should rest assured that there is no cause for concern".

Tim Beardsley

Nuclear waste management

SIR—Robert Walgate (*Nature* 314, 396; 1985) drew attention to work conducted by the University of Surrey on the public's attitudes towards the management of nuclear waste. However, there were some understandable misinterpretations in his report (which was derived from an oral presentation) concerning the issue of compensation. This advance note of some of our results should set the record straight.

Compensation may be seen as one possible mechanism for reducing the social inequity that is inevitable when a local community is required to host a nuclear waste repository to meet a national need (or to conform with national policy).

Our national survey asked 1,511 people "Do you think it would be right to give some form of compensation to people living in an area where nuclear waste is to be buried?", to which 66 per cent replied "yes".

They were asked subsequently whether the compensation should be directed towards the community as a whole or to individuals. Respondents were able to select one or other or both of these suggestions — 48 per cent endorsed personal and 36 per cent community-directed compensation — so it was incorrect to sum these and imply 84 per cent of the public in our survey "could be bought off".

Additional data can put these particular findings into context. People were asked their reactions to proposals to build a range of facilities within two or three miles of their home:

Facility	% Who would move away (if they could)	% Who would actually oppose
Radioactive waste	79	73
Nuclear power station	66	59
Airport	51	32
Prison	47	32
Army barracks	32	16
Motorway	30	17
Industrial estate	21	11

People were also asked about the likelihood of something going seriously wrong

at a radioactive waste site:

Likelihood of accident	% Within 5 years	% Within 50 years
Very likely	10	26
Likely	12	20
Possible	38	28
Unlikely	17	9
Very unlikely	12	6
Don't know	12	12

Finally, respondents picked out those issues that ought to be addressed by a public inquiry. The rank order is as follows:

Rank	Public inquiry issues
1.	Health effect
2.	Long-term reliability
3.	Effects on nature
4.	Suitability of geology
5.	Local opinion
6.	Transport routes and distance
7.	National interest
8.	Land use
9.	Comparative costs of alternative sites
10.	Local employment
11.	Compensation for local community

These findings indicate that radioactive sites are indeed problematic and that from this national sample, health issues are paramount.

Other findings indicate that the sites are likely to generate anxiety not only at the level of "personal safety", but also at the "future character of society" level. Sites are seen as the environmental intrusion most likely to predispose people to move away or protest. However, for many there is neither the luxury to be able to move nor the time or skills to participate actively in the decision process. Compensation is one possible form of mitigation and was certainly not seen as bribery by the majority of our respondents.

T. R. LEE

J. BROWN

J. HENDERSON

C. McDERMID

H. WHITE

Department of Psychology,
University of Surrey,
Guildford, Surrey GU2 5XH, UK

Old hat

SIR—P. W. Hawkes, in his review of J. W. Goodman's *Statistical Optics* (*Nature* 15 August, p.584), states that books on the subject have only recently begun to appear. However, the text *Introduction to Statistical Optics* by Edward L. O'Neill (Addison-Wesley) appeared in 1963. I was a student of Dr O'Neill's at Boston University at about that time, and found his presentation of the ideas of statistical optics fascinating. I would like him to be recognized as one of the early authors in this field.

SYLVIA L. BOYD

PO Box 1050,
Cambridge, Massachusetts 02238, USA

Values in science

SIR—The recent debate about values in science in your columns has surprisingly reached one point of consensus — that values should be made as explicit as possible in scientific procedures. One side of the debate would then exorcise them as far as possible, the other would change them or even introduce others. Since the point of agreement transcends both views, it is itself an example of something as value-independent as possible in this context.

One could derive a semiquantitative figure for the degree to which some experimental result or interpretation is value-independent; perhaps it should be

inversely proportional to the number of researchers with antithetical philosophical, social and religious views able to agree on it. On that basis, science has been surprisingly value-independent. Hence, ironically, at a higher level, it is often intensely valued because such consensus is rare.

Total exorcism of values from science? The demons may always lurk beyond our consensus or even in it. But I thought the consensus principle meant we should exclude such demons as far as possible from the charmed circle — and then deliberately invoke them afterwards from its relative safety?

To paraphrase St Augustine, love consensus, then do as you will.

N.E. WHITEHEAD

International Laboratory of Marine
Radioactivity,
Musée Océanographique,
Monaco-ville,
Principality of Monaco

Selenium-enriched?

SIR—A recent US Geological Survey report (S.J. Deverell *et al.* USGS Water Resources Investigations Report 84-4319, Sacramento, California, November 1984) listed concentrations of selenium found in farm irrigation drain sumps in the San Luis Drain service area on the Panoche Fan. These showed concentrations of selenium as high as 3,800 micrograms per litre.

On 26 July 1985 I filmed tomatoes being harvested in the same area. I am not Paracelsus but I would rather Thomas Jukes (*Nature* 22 August, p.673) ate them than I did.

MICHAEL ANDREWS
(Senior Producer)

Natural History Unit,
British Broadcasting Corporation,
Broadcasting House,
Whiteladies Road,
Bristol BS8 2LR, UK

Chauvinism in the air

SIR—The first sentence in your unsigned Opinion column of 15 August (p. 566) entitled "Geostationary blues" reads: "A World Administrative Radio Conference of the International Telecommunication Union must rank with anything on the subject of Canada as the surest way for newspapers to lose readers". This rather disingenuous statement seems surprising in the pages of the self-proclaimed "International Weekly Journal of Science". More so, since *Nature* has its headquarters in London: one might interpret the aside as the jealous rejoinder of the failing parent to the thriving child.

BARRY T. SMITH

Harvard Medical School,
Joint Program in Neonatology,
75 Francis Street,
Boston,
Massachusetts 02115, USA

Looking for molecular switches

The molecular biology of the process of development has been enlivened, in the past few years, by several novel clues. But there is still a long way to go.

San Francisco

NATURE's conference here last week on genes and systems of development, organized with the generous assistance of the University of California, San Francisco, turned out, perhaps as much by accident as design, to be as good a survey of the molecular biology of development as any. What follows is not a formal report of the meeting, but an inexperienced account of what it now seems possible to say about the way in which organisms acquire different functions as they mature. Ontogeny is the fashionable synonym.

At meetings such as this, the starting assumption is that the genetic endowment of each embryo of every species contains not merely the genes that will function at the several stages of the development of the maturing organism but also those that determine the broad pattern of development, the sequence in which different sets of genes come into play. This does not, however, imply that all organisms are hard-wired and rigorously predetermined as in the nematode *Caenorhabditis elegans*; the observation that the visual system of mammals such as cats remains malleable, and affected by early experience, is a sufficient proof of that. The circumstances in which gross features of earlier stages in development are susceptible to external influences remain, for the most part, to be defined. Little is known of the mechanisms responsible, although it is clear that the development of the nervous system has a high intrinsic interest for other reasons as well.

Outsiders must also learn not to be confused that the pursuit of an understanding of development entails the use of model systems which are very much more particular than the grand name *ontogeny* would suggest are appropriate. That, however, is the spirit in which people at last week's conference (and elsewhere) are concerned with phenomena such as how yeast cells switch between one mating type and another, how white blood cells of different kinds, all descended from the same race of precursor cells, acquire their special properties and how the different kinds of cells in the nervous system differentiate from what is, to begin with, a more or less uniform collection of cells. Development, the argument goes, is a succession of differentiations, steps in which cells become specialized. The objective is to identify the switching mechanism, the genetic switches and the molecules that

actuate them, presumably themselves products of the genes.

This is the sense in which the ever more elaborate, but also elegant, model of lambda-bacteriophage infection of *Escherichia coli* developed by Dr Mark Ptashne (Harvard) is relevant to the issue of the development of much grander organisms. For this is a set of identified genetic switches and actuating molecules, protein molecules in this case, which account for the interaction of the bacterial virus with its host. The obvious question is the extent to which this may be a general model for the switching that occurs during differentiation. Opinion seems to be veering towards the view that the actuator molecules are less likely to be molecules of messenger RNA than protein. But some switches are clearly not on/off switches as with lambda phage but genetic rearrangements. Most probably there will be quite a long catalogue of different kinds of switches before very long.

The great excitement about homoeotic genes in the past year seems an obvious way to extend the present catalogue. The opportunity, now well publicized, is that a set of genes known (from the study of mutations) to determine the body plan of *Drosophila* is distinguished by a certain nucleotide sequence, about 180 basepairs long, which also crops up in other organisms, *Xenopus* (the African clawed toad), mice and also human beings. The inference, but perhaps it is only a hope, is that the "homoeoboxes" that occur in organisms other than *Drosophila* also mark out genetic switches that control the process of development. But it now seems that people are on the point of being able to demonstrate directly, and not merely infer, that the products of the homoeobox-containing genes regulate other genes and constitute components of genetic switches. The more teasing question of what these same genes do in other organisms remains unclear, although the similarity with the switch part of the mating-type genes in yeast is suggestive. No doubt there will also have to be a search for further kinds of genetic switches in other organisms: so far, only five genes with homoeoboxes have been found in the human genome.

Inevitably, these developments (and new technology, such as the easy availability of monoclonal antibodies) have been a stimulus for what might be called the classical embryology of *Drosophila* and other

insects whose larvae consist of segments which carry different undeveloped parts of the mature creature. Here as in other organisms, development at the earliest stages of an embryo appears to be determined not merely by the genes but also by the cytoplasm of the fertilized egg, hence current interest in maternal effects — "maternal" because sperm contributes next to nothing to the yolk. The objective, now, is to tell what kinds of molecules are responsible.

Oncogenes also, but inevitably, play a part. Cells with the carcinogenic forms of oncogenes escape the usual constraints on growth and are developmentally abnormal, so may it be supposed that the proto-oncogenes from which the cancerous forms derive play a part in the regulation of normal development? (Or are they Jekyll and Hyde genes, as the question was put by Dr Michael Bishop (UC San Francisco)?) Now that it seems that the proteins derived from these normal cellular genes are expressed differently in different tissues, and at different stages of development, at least in mice and *Drosophila*, there is some excitement that it may soon be possible to tell how their normal function is regulated, perhaps by molecular influences outside the cell. Much the same is true of the search for the mechanisms by which cells recognize contact with others, and to the presumably molecular influences that guide neuronal projections to the places at which they belong.

So, it seems, there is progress on a broad front towards an understanding of development, largely with the help of several novel techniques and molecular clues (of which the homoeoboxes are only the most dramatic).

But even in the simplest of all the workhorses of development, the presumably hard-wired nematode *C. elegans*, puzzles persist. Why should some cells be programmed to die, for example? And in spite of the attention that has been lavished on the genes known to regulate development in *Drosophila*, there are so many of them, and their functions are so likely to overlap, that it will be a long time before people attempt a full listing of them, with an accompanying chronology showing when they are switched off and on. The obvious difficulty is that such a task is no simpler than that of describing how cells function as biochemical networks. But at least, now, there is movement.

John Maddox

Meteorology

Watery model for microbursts

from Alan Thorpe

AN INGENIOUS laboratory simulation of the microburst — a severe hazard to aircraft on landing and taking off, and the probable cause of the fatal accident at Dallas, Fort Worth Airport in August — is reported by P. F. Linden and J. E. Simpson on page 601 of this issue. In the simulation, a dense fluid (saline water), which represents the cold air flowing out along the ground from a thunderstorm downdraught, flows into a less dense medium, producing a horizontal vortex at the leading edge of the outflow. What is observed is an increase in circulation of the vortex as it decreases in area, when the denser fluid spreads out in three dimensions away from the source. This strong circulation in the vertical plane is likely to be one of the main hazards of microbursts for aviation.

After preliminary studies some 10 years ago by T. T. Fujita of the University of Chicago into extremely localized downdraughts in thunderstorms, several campaigns were set up to observe their properties. These phenomena are now known as downbursts or microbursts if they have a horizontal scale smaller than about four kilometres. Impetus for this research was, and continues to be, given by the well-documented hazards to aviation of downbursts and related phenomena (see Kessler, E. *Nature News and Views* **315**, 179; 1985). Projects, with picturesque acronyms like NIMROD, JAWS and CLAWS, have shown, amongst other features, the existence of the horizontal vortex structure now simulated by Linden and Simpson. In common with most meteorological phenomena, complex flow patterns accompany the real event.

The meteorologist is, however, rarely in the position of the physicist who constructs a controlled laboratory experiment to test theoretical ideas. In a very real sense he is a spectator of the weather, attempting to develop theories about a system whose initial conditions are imperfectly known and certainly uncontrollable. Flows relevant to meteorology that

can be simulated in the laboratory with any degree of realism are therefore of considerable scientific interest. In that respect the work of John Simpson and his collaborators over the past 15 years, using laboratory gravity currents to provide simplified models of thunderstorm outflows and sea-breeze fronts, is notable. Another valuable type of laboratory experiment is the rotating annulus, used to understand the sensitivity of global-scale circulations to basic parameters such as rotation rate, temperature differences between the pole and equator, and variations in surface elevation. In contrast, it is extremely difficult to simulate, for example, a convective cloud in the laboratory as the critical release of latent heat when water vapour condenses to liquid is difficult to reproduce with workable laboratory fluids.

It is important to consider the relationship of these laboratory results to the atmosphere. A thunderstorm is indeed a complex phenomenon from a fluid dy-

namical viewpoint, involving cooperation between an updraught of buoyant, moist air and a cold downdraught. Microbursts are but one aspect of some downdraught outflows; Fujita enumerates many other structures which can be hazardous, including straight-line winds, suction vortices, burst swaths and tornadoes (*J. Atmos. Sci.* **38**, 1511; 1981). Meteorologists are used to describing an entity like a thunderstorm as a whole system, realizing that details of the flow may be dependent on many other related features of the storm. For example, the evaporation and drag that is produced by falling rain is necessary to maintain the storm downdraught, and this air, which feeds the downburst outflow, has both rotation and considerable downward momentum, so may be far from being in hydrostatic balance. The laboratory simulation cannot represent all these properties but can graphically illustrate those aspects of the dynamics of microbursts that are shared with gravity-current flows. A more complete description will come from a combination of observations, laboratory experiments and numerical modelling. □

Alan Thorpe is a Lecturer in the Department of Meteorology, University of Reading, PO Box 239, Reading RG6 2AU, UK.

Developmental neurobiology

Trophic control of channel gating?

from Hans R. Brenner

SYNAPSES are the communication points between nerve cells but do they communicate more than just electrical signals? From work on the development of synapses, the answer is clearly yes. On page 621 of this issue, Marshall¹, now demonstrates that, at neuronal synapses in frog sympathetic ganglia, the dynamic characteristics of postsynaptic ion channels are influenced by the nature of the presynaptic input.

Chemical transmission at synapses requires a high density of receptor proteins in the postsynaptic cell membrane. Binding of neurotransmitters to the receptors ultimately results in an electrical response in the postsynaptic cell. Accumulation of receptors in the postsynaptic membrane seems to be induced by the presynaptic neurone when a synapse is formed. At one specialized synapse, the neuromuscular junction, it is known that accumulation of the receptors is followed by a change in the gating and conduction properties of their ion channels. Therefore, both the number of receptors and their functional properties at this synapse may be influenced by the presence of the presynaptic neurone. Marshall¹ has now shown that the behaviour of postsynaptic channels on certain ganglionic neurones can be altered when the neurones are innervated by foreign axons.

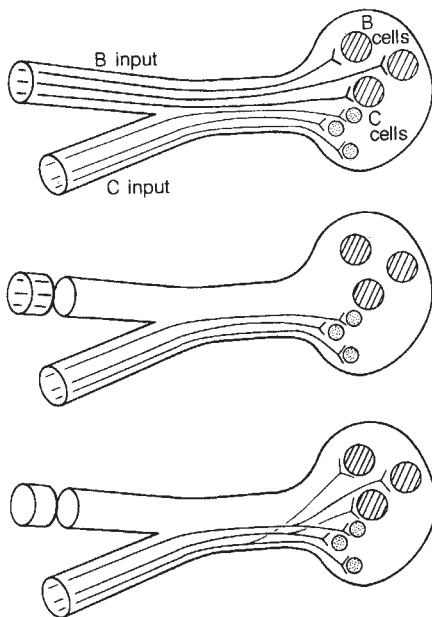
Sympathetic ganglion cells innervate smooth muscle and glands. In frog, two types of principal cells, B and C cells, can be readily distinguished by their size and the velocity with which they conduct electrical impulses. Stimulation of the nerves entering the ganglion, which contains afferent axons innervating the B and C cells (see figure), produces a fast excitatory response followed by slower potential changes in both cell types. The fast excitatory postsynaptic potential is mediated by nicotinic acetylcholine receptors and is analogous to the endplate potential in skeletal muscle. Marshall finds that the channels activated by acetylcholine in the two ganglion cell types differ in the times for which they remain open: the apparent mean open time of B-cell channels is considerably shorter than that of C-cell channels. Furthermore, when B cells are chronically denervated, the gating behaviour of their channels is unchanged but if these cells are reinnervated by the axons that normally go to C cells the open times of the B cell channels are prolonged, becoming similar to those in C cells. This is a strong indication that the expression of specific characteristics of channels in the postsynaptic membrane may depend on the type of presynaptic neurone that innervates them.

The plasticity of the ganglion cells in

IMAGE
UNAVAILABLE
FOR
COPYRIGHT
REASONS

Wreckage of the Boring 747 that crashed in a thunderstorm at Dallas, Fort Worth airport in August 1985 (photograph: Popperfoto).

responding to a change in innervation by altering the kinetic properties of the subsynaptic ion channels seems much more pronounced than that of skeletal muscle fibres. In the frog, when autonomic neurones of the vagus nerve are surgically rerouted to innervate skeletal muscle fibres, the open times of the channels at these mismatched synapses are similar to those of normal muscle endplates². This is much shorter than that of the channels in parasympathetic ganglion cells, which are the normal targets of the vagus nerve. Thus, when skeletal muscle fibres are the postsynaptic cells, the target determines how synaptic channels are gated by responding rather stereotypically to any cholinergic innervation. In contrast, the function of the nicotinic channels in ganglion cells appears to be controlled by the origin of the innervating neurone.



Schematic diagram of the experimental paradigm used by Marshall¹. In the frog sympathetic ganglia, B cells and C cells receive separate inputs (top). The B input fibres were cut and so degenerated (middle). Later the B cells were reinnervated by C-cell inputs, probably by collateral sprouting (bottom).

What is the nature of the presynaptic influence that induces these changes? Some clues come from recent work on the conversion of channels during the development of the neuromuscular junction. Even non-innervated embryonic muscle cells contain two types of acetylcholine receptor (AChR) channels. One of these, a small proportion of the total number of channels, resembles the mature endplates found at neuromuscular junctions³. Conversion to the mature form occurs focally, only where a synapse is formed, suggesting that trophic control by the innervating neurone is involved. There is, however, some indication from the development of surgically induced ectopic endplates in adult rat muscle that muscle activity may be important for endplate differentiation, including the expression of synaptic chan-

nel gating^{4,5}. According to this notion, a neural 'mark' is left on the muscle fibre (see ref. 6) and synaptic channels are expressed at these points following muscle activity. In amphibians, on the other hand, using tetrodotoxin to block muscle activity does not seem to prevent the functional differentiation of the endplate membrane⁷. The relative importance of neurotrophic effects and of activity for synaptic channel expression may thus be different in mammals and in amphibians.

A number of molecular models to explain channel conversion at the developing endplate have been proposed. The two channel types present in the muscle-fibre membrane, junctional and non-junctional, may represent different conformations of the same AChR molecule; or they may be structurally different as a result of synthesis by different genes or posttranslational modification. Recent work suggests the latter⁸. The innervating neurone and the muscle activity it induces would then shift the balance between the two in favour of the junctional type. This could be brought about by a modification of the endplate membrane to favour incorporation of the junctional form, or by the

selective activation of junctional and the suppression of non-junctional AChR channel synthesis.

Similar mechanisms may also be effective in the control of B-cell channels by C-cell inputs in frog sympathetic ganglia. In addition to input-specific neurotrophic influences, different discharge patterns in preganglionic B and C cell inputs may be responsible for the different behaviour of the channels in the two ganglion cell populations and their responses to foreign innervation. A similar interaction has recently been shown for the expression of different molecular forms of acetylcholinesterase at mammalian endplates⁹. □

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Hans R. Brenner is in the Department of Physiology, University of Basel, 4051 Basel, Switzerland.

Dynamical systems

Attraction in a new idea

from Ian Stewart

IN MATHEMATICAL research the emphasis is usually on proving theorems. But it can also be important to find the right setting for basic concepts. For example, it took several centuries to reach an effective definition of 'limit', an idea central to the calculus. In the qualitative theory of dynamical systems, an equally central concept is that of an 'attractor', which describes the system's long-term behaviour. Interest in attractors has grown rapidly since the discovery of a variety of 'strange' attractors which exhibit deterministic chaos. But the literature contains almost as many definitions of the term attractor as there are authors. Examples that ought to be attractors sometimes fail to satisfy the proposed definition, while examples that ought not to be sometimes do. None of this affects the accuracy of the results, but it creates confusion and is obviously an unsatisfactory state of affairs. John Milnor (*Comm. math. Phys.* **99**, 177; 1985) has now proposed a definition that seems to have all the advantages of previous attempts but none of the disadvantages.

Let f be a continuous transformation of a manifold M . Discrete dynamics deals with the effects of iterating f , that is, with the behaviour of sequences $x, f(x), f(f(x)), f(f(f(x)))$, ... for initial points x in M . Milnor's idea is to take seriously an informal approach due to P. Collet and J. P. Eckmann (*Iterated Maps on the Interval as Dynamical Systems*, Birkhäuser, Boston

1980), according to whom the attractor of a map f is "the set to which most points evolve under iteration of f ". Three words in this statement require attention: "most", "evolve" and, because f may have several attractors, "the".

To explain Milnor's approach, a few standard ideas are needed. If x is a point in M , then its omega limit set $\omega(x)$ consists of points in M that lie arbitrarily close to iterates of x under f . A measure on M is a generalization of the ideas of area or volume of sets. Then Milnor defines a closed subset A of a compact manifold M to be an attractor if, first, its realm of attraction $\rho(A)$ — comprising those points x in M for which $\omega(x)$ is contained in A — has non-zero measure and, second, there is no smaller set B for which $\rho(B)$ coincides with $\rho(A)$, except for a set of measure zero. As Milnor explains: "The first condition says that there is some positive possibility that a randomly chosen point will be attracted to A , and the second says that every part of A plays an essential role".

The use of the omega limit set in the definition of the realm of attraction takes care of "evolve". The second condition deals with "most". The fact that only $\rho(A)$ is attracted to A allows for more than one attractor, each with a different realm of attraction. Of course, this definition has close resemblances to many previous proposals. The set $\rho(A)$ is known in the

literature as the basin of attraction if it is an open set, and as the stable manifold if it is a smooth manifold of dimension smaller than M . But Milnor's realm of attraction need be neither open nor a smooth manifold, so it is more general.

The definition has many nice features. First, every f has at least one attractor. To prove this, Milnor introduces the likely limit set of f , and shows it is the unique largest attractor of f . Again, any compact set of positive measure that is mapped into itself by f must contain an attractor. Most important are the minimal, or smallest, attractors. There may be an infinity of these, but it is a countable infinity. Some maps f may have no minimal attractors; the identity map, which transforms every point of m into itself, is a trivial example. And if this likely limit set breaks

up into a finite number of minimal attractors, their realms of attraction in m will divide this manifold into a finite number of sets of positive measure except for one set of measure zero.

Milnor discusses a number of important features of dynamical systems, such as stability and robustness, showing how these relate to his definition. He also analyses a number of key examples from the new point of view, showing that they fit naturally into the proposed framework. Perhaps now workers in the field of dynamical systems will be able to agree on the interpretation of one of the most basic concepts of the subject. □

Ian Stewart is in the Mathematics Institute, University of Warwick, Coventry CV4 7AL, UK.

Positron emission tomography

Progress in brain imaging

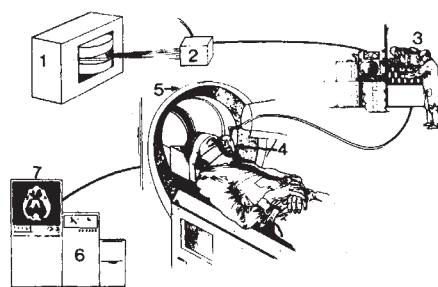
from Marcus E. Raichle

MORE than a decade has passed since the introduction of positron emission tomography (PET), a technique that can be used safely to measure physiological and biochemical processes in the brain and other organs of human subjects *in vivo*. Large amounts of national research funds have been spent in equipping a small number of laboratories around the world with the imaging equipment, cyclotrons, chemistry laboratories and staffs of highly skilled professionals (see figure) needed to accomplish the proposed studies. Has PET so far justified such an expenditure?

Emission tomography is a technique that produces an image of the distribution of a previously administered radionuclide in any desired section of the body. PET utilizes the unique properties of the annihilation radiation generated when positrons are absorbed in matter to provide an image that is a highly faithful representation of the spatial distribution of radionuclide at a selected level in the tissue'. The images are effectively equivalent to quantitative tissue autoradiograms obtained with laboratory animals but PET has the added advantage that it is non-invasive, hence studies are possible in living animals, including humans. The complexity of the PET method arises in part from the short half-life of the most commonly used radionuclides: about two minutes for ^{15}O , 10 minutes for ^{13}N , 20 minutes for ^{11}C and 110 minutes for ^{18}F . This means that an in-house cyclotron and skilled chemists are necessary to incorporate quickly the radionuclides into appropriate radiopharmaceuticals.

Using ^{18}F -labelled fluoro-deoxyglucose, PET investigators quickly adapted the successful deoxyglucose autoradiographic technique developed by Sokoloff *et al.*² for measuring local brain-glucose metabolism

so that PET tended to become synonymous with deoxyglucose measurements of local brain-glucose metabolism in humans. Many attractive, colour-coded images of normal, as well as diseased, human brains at work soon appeared in the scientific literature, at scientific meetings and even in the media. This highly visible beginning fuelled premature expectations of early scientific and clinical payoffs from an expensive and very com-



PET study of regional cerebral oxygen utilization. A beam from a cyclotron (1) hits a target (2) to produce ^{15}O , which is fed to the control console (3), where a radiochemist monitors the gas supplied to the patient through a mask (4). An array of detectors (5) record the positron captures, which are processed by computer (6) to reconstruct an image of the section on the screen (7). (Drawing courtesy of T. Jones.)

plex new technology. It also raised doubts about the validity of the deoxyglucose technique^{3,4,5}, misinterpreted by some to indicate that PET itself might be invalid⁶.

The controversy surrounding the deoxyglucose technique itself has now largely been resolved^{7,8}, with the result that this very interesting and important technique is now much better understood, and there is a greater respect for the complexity of PET. What escaped the

notice of the critics was that PET employs a variety of quantitative tracer techniques in addition to the deoxyglucose method each utilizing a different mathematical model and radiopharmaceutical. These techniques can be used to make such diverse measurements as local blood flow⁹, blood volume¹⁰, oxygen consumption¹¹, pH (ref. 12), blood-brain barrier permeability¹³ and receptor binding¹⁴.

Using accepted techniques for the measurement of brain blood flow and metabolism, PET has provided important new information in the metabolism of specific brain tumors¹⁵, the effect of focal epilepsy on the brain¹⁶, and the changes in local metabolism that occur with aging¹⁷, in dementia¹⁸ and in certain other degenerative diseases¹⁹. Equally promising are studies of the pathophysiology of strokes as well as the identification of patients at increased risk for stroke²⁰. Studies of major psychiatric diseases, like schizophrenia, mania and depression, have received considerable attention but, for the most part, results have been conflicting and inconclusive. This probably reflects difficulties in establishing reliable diagnoses, failure to account for factors such as the effect of medications and to do well controlled, quantitative measurements. A report last year in *Nature*²¹ indicates, however, that when a well-characterized and clearly validated PET measurement is applied to a well-defined subject population, psychiatric disease will become a very fruitful area for investigation with PET.

PET has also demonstrated its capacity to contribute to a better understanding of normal brain function. Abundant evidence indicates that functional activity, for example, somesthesia, audition, movements of all types and vision, causes striking changes in local brain blood flow and glucose uptake, which can be quite precisely detected with PET. Analysis of such changes has progressed from simple qualitative, uncontrolled demonstrations of anticipated changes — 'gee-whiz' images — to much more sophisticated studies using rigorously controlled experimental paradigms and precise analytical techniques²². It might be argued by some that it will be virtually impossible for PET to reveal the underlying neuronal elements participating in such changes, and hence contribute little to our understanding of how the brain works. It seems fair to assume, however, that once PET has safely identified a specific area of normal human cortex responsible for a well-defined type of information processing, a task it is uniquely equipped to do, other neurobiological techniques can be brought to bear on the exact nature of the process. Complementary interaction of this type will benefit both clinical investigators and basic neuroscientists.

A new and important area of PET research is neuropharmacology. Most attention has been directed to the

development of quantitative techniques for the measurement of receptor pharmacology^{12,13}. This work was stimulated by many elegant demonstrations of binding of specific radioligands to receptors in tissue homogenates or slices.

The transition from quantitative *in vitro* binding studies to *in vivo* examination with PET is complex. *In vitro*, binding affinity and number of binding sites are analysed under strict equilibrium conditions which are easily achieved. *In vivo* studies have to be made under non-equilibrium conditions brought about by factors which affect the regional concentration of free radioligand in the brain, such as nonspecific binding of tracer in blood and brain, blood-brain barrier permeability and blood flow. The metabolism of the tracer can also cause various radiolabelled metabolites to appear in both blood and tissue. As a result, quantitative estimates of the number of receptors and their affinity cannot be obtained from a single measurement; rather, multiple sequential measurements, easily obtained with PET, are needed to delineate

the time course of the radioactivity both in the tissue of interest and in the blood, with a correction for the presence of labelled metabolites in the blood.

The data must then be analysed using an appropriate mathematical model to relate brain and blood radioactivity measurements to measurements of receptor physiology. Inferences drawn from qualitative, *in vivo* measurements based on single tissue autoradiograms or PET images must be viewed with extreme caution, despite their intuitive visual appeal. Unfortunately this sort of inference is the rule rather than the exception.

Recent attempts to examine the more complex but equally interesting subject of transmitter metabolism with PET have suggested another fruitful area of research¹⁴. All the complex issues facing the study of receptor pharmacology also apply here, with the added problem that local tissue metabolism of the radiolabelled tracer is especially critical. Considerable time, effort and money will be necessary to perfect such measurements but current evidence suggests they

will be amply rewarded.

A thoughtful review of PET research clearly indicates it is gradually fulfilling its promise as a legitimate and important tool for clinical research. Considerable unevenness in the quality and quantity of published research from extant PET centres is, however, very evident. What are some of the problems? One factor is clearly a normal process of maturation of a very complex technology, one which is simultaneously advancing on several important fronts. Initial development is necessarily slow — particularly as instrument design and construction, radio-pharmaceutical development and tracer modelling, as well as experimental design, execution and interpretation have often all had to occur in a single PET laboratory. For success, this process requires interaction and close collaboration between investigators with expertise in instrumentation, chemistry, tracer kinetics, clinical neurology and neurobiology: full commitment is essential. Workers in this field would now agree this is not easily or quickly achieved, which perhaps accounts

John F. Enders (1897–1985)

JOHN ENDERS, who died unexpectedly on 8 September 1985 just as he had finished reading a volume of poems by T.S. Eliot, was awarded a Nobel Prize for Physiology and Medicine in 1954 for discovering how to grow the poliovirus in culture, which was crucial to the development of the first polio vaccine.

Enders had an almost lifelong scholarly interest in English and Celtic literature, and it was while working on a doctoral thesis about the use of gender in Middle English that he became interested in microbiology. He was sharing rooms with H.K. Ward, who had come from Australia to work with Hans Zinsser and Enders's long-latent interest in biology was roused by his visits with Ward to Zinsser's laboratory. Consequently, when he did obtain a Ph.D. from Harvard University in 1930, his thesis concerned the purification from the tubercle bacillus of the carbohydrate antigen that caused anaphylaxis and was separable and distinct from the protein antigen that caused delayed hypersensitivity. Like most microbiologists of his generation, Enders studied the tubercle bacillus and the pneumococcus in the search for improved serum therapy. In 1933, his classic paper with Ward showed that the opsonic activity of serum against type II pneumococci was due to specific antibody and complement.

His interest in virology resulted from a devastating epizootic in kittens that swept through the animal quarters of the Harvard Medical School in 1937. He quickly discerned that the cats had a disease that a filterable agent could transmit. His publication in 1939 on distemper in cats must be a landmark in the clinical feline liter-

ature for it describes in clear and concise detail the clinical course, the pathology and the aetiology of the disease. He became interested in the problem of *in vitro* propagation of viruses and, in 1940, reported with A.E. Feller and T.H. Weller the first successful prolonged culture of a virus in roller-tube cultures. These promising leads on the cultivation of vaccinia virus, and subsequently influenza virus, were interrupted by the outbreak of the Second World War. In 1941 he became a consultant to the Secretary of War on epidemic diseases. A massive plasma fractionation programme was being developed in E.J. Cohn's laboratory at the Harvard Medical School and Enders showed that antibodies to a variety of antigens were not fractionating uniformly. He anticipated the future discovery of IgM, IgA and IgG antibodies. At the war's end, he took up a detailed study of the mumps virus; he purified the complement-fixing antigen from parotid glands of infected monkeys, developed the skin-test material for eliciting delayed hypersensitivity in immune individuals and eventually he managed to propagate the mumps virus in tissue culture.

While this work was progressing, he moved his laboratories from the Department of Microbiology at the Harvard Medical School to the nearby Children's Hospital. There he was joined by T.H. Weller and F.C. Robbins. In 1949, they jointly reported in *Science*, in the briefest and most understated terms, the sensational finding that they had succeeded in propagating the poliovirus in tissue cultures of non-neuronal origin. They measured a 10¹⁵-fold increase in infective virus

during the period of culture as assayed by mouse intracerebral inoculation. Within the year, they were able to circumvent this cumbersome bioassay by observing a cytopathic effect on human foreskin fibroblasts *in vitro* as revealed by changes in pH in the culture fluid. In three brief, concise reports, the groundwork was laid for the development of a vaccine that has relieved untold amounts of human deformity, suffering and death. In 1954, Enders was awarded the Nobel Prize for this discovery but declined to accept the honour unless his two young colleagues, Weller and Robbins, who, as he said, "had done the work", could also share the prize. And so they did. This must be the most unselfish act in the history of the Nobel Prize and betrays the humbleness, decency and modesty which characterized the life of John Enders.

In the following summer, ironically, Boston was struck by the most devastating polio epidemic in its history. By this time others were busily engaged in polio vaccine development, so Enders turned his attention to the measles virus and successfully developed a measles vaccine. In his last decade of work, he made important contributions to the transformation of cells *in vitro* by simian virus 40 and the oncogenicity of these transformed cells in hamsters. He finally ended his active work in 1972 at the age of 75.

John Enders was in many ways a richly endowed man and he shared these gifts with many people. He was descended from a family that had great success in the insurance and banking businesses but he eschewed a life of ease, or one in the financial world, after a brief and unsuccessful attempt at it in 1920. He felt that his roots lay with Edward Jenner and Louis Pasteur, whose picture always adorned his office. In this he was correct.

F.S. Rosen

for the relatively small number of high quality PET centres in operation.

A review of the PET literature also reveals studies with two very different goals. One type is designed to evaluate the usefulness of PET as a clinical tool, whereas the other attempts to answer well formulated research questions. This dichotomy reflects the expectations of the investigators involved in a particular study and the requirements, which are very different. Quantitative accuracy, although desirable, is not really necessary for a technique to be clinically useful, whereas it is essential for research. The ultimate clinical benefit of any diagnostic procedure is its ability to reduce morbidity and mortality by providing information not previously available or by replacing more expensive or hazardous procedures. Thus, each study must be judged on its intent but this is, unfortunately, not always clear.

Will PET be superseded by nuclear magnetic resonance (NMR) imaging, which should ultimately enable us to carry out spectroscopy and make biochemical measurements *in vivo*? The answer is no. PET, with its far superior sensitivity²⁵, will continue to employ classic tracer techniques to provide unique information on an increasingly wide range of physiological variables including many, such as receptor pharmacology and neurotransmitter metabolism, that are simply not possible with NMR. The majority of NMR studies to date involve high resolution anatomical imaging, not spectroscopy. NMR spectroscopy will undoubtedly complement PET by providing *in vivo* information on such things as metabolic intermediates and tissue electrolytes, albeit at a much

lower sensitivity than PET and, hence, with somewhat lower resolution.

For PET to achieve its true potential it must blend into the fabric of biological science, seeking enrichment from the vast storehouse of new information available from the basic sciences. This can only occur through direct, personal interactions among fully committed individuals at the forefront of science. PET will then contribute, effectively and uniquely, to a better understanding of human biology. □

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Marcus E. Raichle at the Washington University Medical Center, St Louis, Missouri 63110, USA.

Chemistry

Cyclic fixation of nitrogen

from Fraser Armstrong

THE catalytic fixation of nitrogen to produce ammonia at ambient temperatures and pressures in an electrochemical cell, reported on page 652 of this issue, is a considerable achievement in basic chemistry and has interesting similarities to nitrogen fixation as occurs, for example, in plant root nodules.

It is widely believed that nitrogen fixation by nitrogenase is catalysed by a co-factor that contains molybdenum, iron and sulphur and to which electrons are conveyed by iron-sulphur clusters. While the detailed molecular mechanism has yet to be established, insight has been gained by studies of viable chemical pathways based on reactions of structurally characterized small molecules. A primary consideration has been the accommodation of simultaneous demands for protonation and electronation, reflected in the stoichiometry of ammonia formation: $N_2 + 6H^+ + 6e^- \rightarrow 2NH_3$.

As a free molecule, dinitrogen is chemically rather inert. However, since the discovery of the first metal dinitrogen complex $[Ru(NH_3)_5(N_2)]^{2+}$ by Allen and Senoff (*Chem. Commun.* 221; 1965), the possibility that dinitrogen could be activated via coordination to electron-rich metal atoms has been pursued with interest. In 1975, J. Chatt and coworkers at the Agricultural Research Council Unit of Nitrogen Fixation at the University of Sussex showed that dinitrogen bound in monomeric molybdenum or tungsten complexes of the type $[M(N_2)_2(PR_3)_4]$, where M is Mo or W and R is an alkyl or aryl group, could be activated towards protonation to yield ammonia in near-theoretical amounts (*Nature* **253**, 39; 1975). The reaction of these complexes with sulphuric acid in methanol produced two molecules of ammonia and one molecule of dinitrogen, and demonstrated chemical guidelines for achieving the above reaction

under ambient laboratory conditions. But the extension to a full catalytic process, analogous to enzymatic nitrogen fixation and suitable for development as a viable means for ammonia synthesis, was restricted by oxidative degradation of the core $[M(PR_3)_4]$. This occurs because the necessary electrons are formally derived from the metal, without any external replenishment, and dissociation of the monodentate phosphine ligands and substitution by sulphate occurs as the formal metal oxidation state is increased.

As C.J. Pickett and J. Talarmin now report, chemists at Sussex have devised a catalytic scheme whereby protonation of tungsten diphosphine dinitrogen complexes is coupled with electrochemical reduction in a nitrogen atmosphere to yield ammonia, with regeneration of the starting material $trans-[W(N_2)_2(dppe)_2]$, where $dppe$ is 1,2-diphenylphosphinoethane. By using bidentate phosphine ligands, dissociation is prevented and an intact metal core is conserved. The development is particularly interesting since certain intermediate species have been well-characterized in independent studies.

Stoichiometric proton transfer to $trans-[W(N_2)_2(dppe)_2]$ from two equivalents of added tosylic acid (toluene *p*-sulphonic acid) yields the hydrazido(2-)(=N-NH₂) complex, for which the formal oxidation state of tungsten has been raised from 0 to IV. The remaining dinitrogen ligand is replaced by tosylate, giving $[W^IV(NNH_2)(OTs)(dppe)_2]^+$. Chatt and his coworkers had observed that further protonation of hydrazido(2-) complexes, leading to ammonia formation, did not occur unless electron density at the metal could be replenished. With complexes having four monodentate phosphine ligands this could be achieved through replacement of phosphine by sulphate, a much stronger donor. Metal complexes with two bidentate phosphines are, however, more resistant to substitution, thus protonation is terminated at the hydrazido(2-) stage. The chemistry of this class of compounds, in particular the dialkyl derivatives (=N-NR₂), has received much attention. Most interestingly, it has been found that the complex $trans-[Mo^IVBr(NNR_2)(dppe)_2]^+$ can undergo two-electron reduction with loss of bromide to yield a reactive five-coordinate species $[Mo^II(NNR_2)(dppe)_2]$ (Pickett, C.J. and Leigh, G.J. *JCS chem. Commun.*, 1033; 1981). Treatment with anhydrous HBr yields the amine HNR₂ and an imido complex $[Mo^IVBr(NH)(dppe)_2]^+$ (Hussain, W., Leigh, G.J. and Pickett, C.J. *JCS chem. Commun.*, 747; 1982). Thus the requirement for ligand substitution, to maintain electron density on the metal, can be superseded by a clean reduction step which conserves the $M(dppe)_2$ equatorial configuration.

Pickett and Talarmin suggest that ammonia formation proceeds by protonation and the resultant N-N bond cleav-

age at the analogous reactive complex $[W^{IV}(NNH_2)(dppe)]$, afforded by electrochemical reduction of $[W^{IV}(NNH_2)(OTs)(dppe)]^+$. An important point to note here is that tosylate, as an excellent leaving group, facilitates the necessary reductive transformation from six- to five-coordination with conservation of the $=N-NH_2$ entity. The second ammonia is suggested to arise by rapid reduction and protonation of the resulting W^{IV} imido complex, although this and subsequent intermediates are not detected, indicating that the relevant sub-processes are not rate-limiting. The overall proton requirement is met by sacrificial deprotonation of $[W^{IV}(NNH_2)(OTs)(dppe)]^+$, thus permitting protonation reactions to accompany low-potential electrolysis without troublesome levels of hydrogen evolution.

There is indirect evidence that a hydrazido(2-) ligand bound at a single metal site is an intermediate in the nitrogenase cycle (Thorneley, R.N.F., Eady, R.R. and Lowe, D.J. *Nature* 272, 557 1978). This suggestion hinges largely on the observation that chemical quenching of pre-steady-state enzyme turnover produces the same yield of hydrazine regardless of whether an acid or alkali quench is used. Of the tungsten phosphine complexes with various bound dinitrogen hydride species, only hydrazido(2-) complexes also give hydrazine in equal yield from acid or alkali hydrolysis. Free hydrazine, itself, is a poor substrate for ammonia formation, so is unlikely to be

an intermediate compound.

Both enzymatic and electrochemical generation of ammonia involve low-potential species which can reduce protons that, necessarily, are available. Thus nitrogenase turnover faces competition from hydrogen evolution. Lowe and Thorneley (*Biochem. J.* 224, 877; 1984) suggest that the competition is minimized by step-wise electronation and protonation. The consequent slow turnover rate ($1.5 s^{-1}$), which is compensated for by high cellular concentrations of the enzyme (up to 40 per cent of the cytoplasmic protein) reflects the price that nitrogen-fixing organisms have to pay for producing ammonia rather than hydrogen. This problem has not been completely overcome in Pickett and Talarmin's system, in which temporal separation of protonation and electronation is still required.

There remains the question of how dinitrogen interacts chemically with the enzyme and is transformed into ammonia; the important structural aspects are as yet undetermined. The demonstration of a cyclic route to ammonia synthesis through several identifiable tungsten phosphine complexes has attractive technological possibilities. It must also be seen as a valuable elaboration of a working chemical framework within which some features of nitrogenase activity are evident. □

Fraser Armstrong is a Royal Society University Research Fellow at the Inorganic Chemistry Laboratory, Oxford University, South Parks Road, Oxford OX1 3QR, UK.

Conservation biology

Future of the world's primates

from Jared M. Diamond

PRIMATE conservation poses paradoxes. Although our own numbers continue to grow, almost all other primate species are declining, many of them verging towards extinction. Yet it would seem relatively easy to enlist support for saving primates: they exceed all other animals, except perhaps pandas and big cats, in their popular appeal; they play a unique role in biomedical research; and they exist in varied societies and are often ecologically dominant, making them of interest to biologists. But that same demand for primates, combined with their low population densities and reproductive rates, makes them vulnerable. These paradoxes were explored at a recent meeting*, at which many speakers summarized the status of the approximately 200 primate species in the world.

Most species live in tropical forests, with the highest concentration in Brazil (51 species), followed by Zaire, Cameroon, Madagascar, Peru, Colombia and

Indonesia (about 30 each). Of the 13 species of apes, all except four gibbon species are now endangered. There are less than 400 mountain gorillas, and no population of pygmy chimpanzees is protected by any national park. All Madagascan primate species are declining. In the Atlantic forest of Brazil, which has been cut to less than 5 per cent of its former area, 14 out of 21 primate species are endangered. These include the woolly spider monkey (*Brachyteles arachnoides*), the largest New World primate, now reduced to about 240 individuals surviving from a former 400,000; and the golden lion tamarin, down to 200–300 wild individuals in a single forest patch of 20 square miles. Even the widely distributed rhesus macaque, the primate species that is most tolerant of humans, has declined to about 3 per cent of its former numbers in India. When such an abundant, hardy and adaptable species is so badly affected, how can rare and shy forest-dwellers be protected?

The ideal and cheapest solution would be conservation in the wild. Reserves designed around popular primates as 'flag-

ship species' save thousands of other plant and animal species. As presentations by David Chivers, Russell Mittermeier, John Oates and Amy Vedder illustrated, people in underdeveloped tropical countries will support primate conservation only where there are massive educational campaigns combined with economic incentives. In Rwanda (the African country with the highest human population density), the local people other than the poachers knew almost nothing about mountain gorillas in 1978. As a result of a comprehensive project focusing on the needs of local people, poaching had virtually ceased by 1984, and tourism based on viewing wild gorillas became the country's third-ranked earner of foreign currency.

The drawbacks of the alternative solution of captive preservation in zoos — expense, genetic impoverishment, destruction of learned behavioural traditions, diversion of attention from wild primate populations and no benefits trickling down to conservation of thousands of other species — were summarized by David Western. For instance, the last 14 northern white rhinoceroses remain in the wild in Africa, at continued risk of



Logo of World Wildlife Fund campaign to save the cotton-top tamarin in Colombia.

poaching. They would be safer in zoos but African governments refuse to permit their capture, correctly foreseeing that foreign financial support for rhino habitat would end if the animals were in zoos.

Nevertheless, the tragic litany of extinct species illustrates that captive preservation must sometimes be considered, if only as a fail-safe strategy. Endangered non-primate species that have been or soon may be reintroduced to the wild from captive populations include the nene, Arabian oryx and Przewalski's horse; primates likely to join this list include the golden lion tamarin (discussed by Benjamin Beck and Devra Kleiman of the US National Zoo), ruffed lemur, lion-tailed macaque and spider monkeys. A further justification for preservation in captivity is that many studies of behaviour and physiology require captive animals. Finally, without the public interest, and hence

* "Primates: the Road to Self-sustaining Populations", sponsored by the San Diego Zoological Society and the Morris Animal Foundation, 24–28 June 1985 in San Diego, USA.

money, that captive animals evoke, there would be much less money for preservation in the wild.

How successful has captive breeding of primates been? Marvin Jones, Jonathan Pollock and Nate Flesness explained that many of the biological problems have been solved: 127 of the world's 200 primate species have bred in captivity. But for only 46 of those species do the records of the International Species Inventory System indicate a self-sustaining zoo population exceeding 100 individuals. Worse, for only eight species does the effective population size, corrected for sex ratio, exceed 100. Thus, captive breeding

is estimated at 43,000 animals per year, with the United States the leading importer, followed by Britain, France, Japan and Taiwan (*The International Primate Trade* Vol. 1; eds Mack, D. & Mittermeier, R. Washington DC, 1984). For each animal imported, another dies during capture or in transit. The biomedical community has not been heavily involved in primate conservation or in monitoring the effect of the primate trade on wild populations: it has simply shifted its supply sources as successive countries imposed export bans. For instance, the United States currently imports 7,000 long-tailed macaques annually from the Philippines, but there no census

primate conservation, the Primate Programme of the World Wildlife Fund of the United States, whose total annual budget covering many projects is about \$250,000. Budgets of the largest conservation projects directed at many other species as well as primates, such as the annual budget of Serengeti National Park (\$100,000) or of Brazil's Atlantic forest programme (\$125,000), are equally modest. Annual budgets of other primate conservation projects can be less than \$1,000 per year. For each such programme there are many other equally worthy ones that could not be funded. For the cost of one captive-bred rhesus, Madagascar could double its national park budget. For one-seventh of the United States annual expenditure for importing long-tailed macaques, Cameroon could proceed with setting up three new national parks to protect 26 primate species.

It would be wrong to conclude that the biomedical community has a good deal and only primates and conservationists have a bad deal. As the conference illustrated, the current solution that relies on captive breeding plus largely unmonitored cropping of wild animals is a bad deal for biomedical research as well, in at least three ways. First, captive-bred animals cost much more than imported wild ones (for example \$1,000 for a captive-bred rhesus macaque in the United States, \$2,000 in Holland, against \$200–300 for the similar long-tailed macaque caught in the wild). An NIH-funded study at Iquitos (Peru) showed the feasibility of sustained cropping of wild tamarins without depleting their numbers. Second, the present situation cannot last: most primates used in research are still caught in the wild, most wild populations that have been studied are declining and there is a trend towards export bans by more and more countries with wild primates. Finally, vanishing wild stocks mean that the possibility of establishing self-sustaining captive populations of more primate species is also vanishing. It takes many years of continuing wild primate importation to establish a captive colony: for the golden lion tamarin, it took eight years for the colony size to rise steeply and 13 years to reach even the present modest size of 400 animals. Thus, although we know that different species are suited for studying different problems, the present limited suite of captive primate species available for biomedical research is unlikely to increase.

Thus, an increase in the present shoe-string funding levels for primate conservation might be cost-effective for biomedical research in decreasing animal costs while increasing supply stability and diversity. It would also benefit primate conservation and, indirectly, the conservation of many other species. □

Jared M. Diamond is Professor of Physiology at the University of California Medical School, Los Angeles, California 90024, USA.

Comparative costs of primate conservation and of primate breeding for biomedical research.

	\$
Cost of a young pygmy chimpanzee (an endangered ape species) in Zaire	16
Annual salary of graduate biologist in Indonesia's Conservation Department	180
Annual budget (excepting salaries) of Madagascar's National Reserve system	900
Cost of a typical primate project in developing countries, funded by WWF†	
Primate Action Fund	500–3,000
Annual budget of first modern long-term study of wild lemurs, recently begun in Madagascar	5,000
Annual budget of Serengeti National Park (one of the largest and most successful tropical national parks)	100,000
Annual budget of WWF programme to save Brazil's Atlantic forests	125,000
Annual worldwide budget of WWF Primate Programme	250,000
Annual maintenance cost of chimpanzees used for research in the United States (1000 animals, \$5000 per animal)	5,000,000
Annual purchase cost of captive-bred rhesus macaques used in the United States (6000 animals, \$1000 per animal)	6,000,000

† WWF, United States office of the World Wildlife Fund.

to date has rarely achieved the conference's goal of providing a self-sustaining, safely large population. The main obstacles have been lack of funds and public opposition to the capture of wild primates. To postpone captive breeding efforts until the wild population is endangered guarantees competition between wild and captive preservation. The resulting disputes, exemplified by the current debate over what to do with the last nine wild California condors, could be avoided if captive breeding were started in parallel with preservation in the wild before a species becomes endangered.

Primates are essential in biomedical research because they spontaneously develop or can be inoculated with many human diseases such as polio, malaria, acquired immune deficiency syndrome (AIDS), kuru, yellow fever, Down's syndrome, colon cancer and diabetes mellitus. The development of the polio vaccine consumed over a million rhesus macaques; the hoped-for development of vaccines against AIDS and malaria would be just as unthinkable without primate testing. Thus the availability of other primates is literally a matter of life or death for *Homo sapiens*.

Initially, the need for primates in research was met wholly by wild animals. Imports of rhesus macaques to the United States for polio research peaked in the late 1950s at 200,000 animals per year. Even today, the world trade in primates is est-

imated at 43,000 animals per year, with the United States the leading importer, followed by Britain, France, Japan and Taiwan.

As wild primate populations dwindled through the 1970s and public opposition to the primate trade grew, the National Institutes of Health (NIH) could foresee growing jeopardy to wild supplies. (India banned the export of rhesus macaques in 1978.) The NIH solution, reviewed by William Gay and Dennis Johnsen, was to support captive breeding, particularly of rhesus but also of other species. About 55,000 primates are used annually in research projects in the United States alone. A captive-born adult rhesus macaque can be bought in the United States for roughly \$1,000, while a captive chimpanzee can be maintained for about \$5,000 per year. These costs may seem huge compared with costs of laboratory mice, but they constitute less than 15 per cent of NIH research grant budgets and are not a crippling burden on biomedical research.

Dilemmas arise, however, when one contrasts these expenditures for captive-bred primates used in research with expenditures for primate conservation. The table compares two components of expenditure in the United States for captive-bred primates against the costs of some conservation projects in the wild. Although these two components (each around \$5 or 6 million annually) represent only part of the biomedical primate expenditure in only one country, they dwarf even the largest existing programme in

Plant genetics

Transformation of cereal crops by direct gene transfer

from M. G. K. Jones

ONE problem in using recombinant DNA technology to engineer genetically cereals (the most important food crops) and other Gramineae has been the inability of *Agrobacterium* to act as a gene vector for such plants, despite its success in dicotyledons. But it has now been demonstrated that transformation of gramineous protoplasts, with the production of genetically modified callus tissue, can be achieved by direct gene transfer without the need for *Agrobacterium*-based vectors^{1,2} by extending earlier successful experiments that used tobacco protoplasts³. Mendelian inheritance of genes directly introduced into tobacco, following crossing of regenerated plants, has also been demonstrated^{4,5}. The work shows that there is no fundamental molecular block to transformation of gramineous species and indicates that genetic engineering technology will soon be brought to bear on cereal crops.

For dicotyledonous species, methods of gene transfer into plants using *Agrobacterium* can be used routinely to achieve stable integration of foreign genes into the nuclear genome; the transfer of such genes to offspring following sexual crosses has been monitored. Attempts to use the same approach for monocotyledonous species have met with some success, as described in these columns⁶. Thus, *Narcissus* and *Chlorophytum* produce swellings that contain opines following inoculation with *A. tumefaciens*⁷ — expression of opine synthesis genes usually depends on their transfer into plant cells. And the induction of tumorous growth in *Asparagus* plants by *A. tumefaciens* with the production of nopaline⁸ demonstrates that some monocotyledonous plants are susceptible to infection by *Agrobacterium* and can express tumour DNA, although conclusive proof of the integration of foreign DNA is lacking.

In the earlier work on tobacco (*Nicotiana tabacum*) Paszkowski, Potrykus and co-workers introduced selectable foreign DNA into protoplasts by direct gene transfer, where it became integrated into the nuclear genome. This DNA is stably incorporated and is transmitted to progeny plants in a mendelian fashion^{3,4,5}. The direct gene transfer approach has been used routinely to transform animal cells; it is surprising that this method has not been more widely attempted for plant cells. One reason is that plant protoplasts, required for the method, are more fragile and more difficult to culture than many animal cells. In particular, as a result of the trauma of isolation, plant protoplast DNA synthesis and mitoses are usually

delayed for some days following isolation. Integration of foreign DNA may occur during genome DNA synthesis, so the introduced DNA may be degraded by nucleases before it can be integrated.

In the work on gramineous protoplasts, Lorz and co-workers introduced DNA from a selectable plasmid (pBL1103-4, containing a selectable chimaeric gene with the nopaline synthetase promoter, the coding region of Tn5 aminoglycoside phosphotransferase type II gene, the polyadenylation signal of the octopine synthase gene and a maize transposable element, Ac) into protoplasts isolated from suspension cultures of *Triticum monococcum*¹. Transformed cells were selected and characterized by detection of aminoglycoside phosphotransferase II activity. The transformation frequency was about 1 in 5×10^5 protoplasts. The Ac element was included in the introduced DNA to study its possible activation after integration in a cereal other than maize.

Potrykus and colleagues² used the same selectable plasmid (pABDI) that they had used previously to demonstrate direct gene transfer in tobacco. This consists of a chimaeric construction in which the

aminoglycoside phosphotransferase gene of transposon Tn5 is under the control of promoter, terminator and initiation signals from gene VI of cauliflower mosaic virus. In this case the protoplast recipients were obtained from suspension cultures of *Lolium multiflorum* (Italian ryegrass), and cell colonies resistant to antibiotic G418 were selected (untransformed ryegrass suspension protoplasts were resistant to relatively high concentrations of kanamycin). The active product of the phosphotransferase gene was found in these selected cells. In addition, genomic DNA from these lines was extracted and probed with the nick-translated gene by Southern blot analysis, and the presence of at least five copies per genome of the introduced DNA was detected, apparently without any further rearrangements. Thus the stable integration of a foreign gene into a gramineous species has been achieved. In this case the transformation frequency is about 1 in 4×10^5 .

Similar methods were used in both cases to introduce the DNA into the protoplasts. Following linearization of the plasmid DNA, its introduction into the protoplasts was accomplished by incubation of protoplasts and DNA in the presence of polyethylene glycol with or without excess carrier DNA, using procedures developed by Krens *et al.*⁹ who transformed dicotyledonous protoplasts directly with Ti-plasmids. In the work with *Lolium*, the protoplasts were also subjected to a heat-shock stage (45°C for 10 minutes followed by 10 seconds at 0°C) before undergoing

IMAGE
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FOR
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REASONS

Skeletons of dwarf elephants *Elephas falconeri* from Spingallo cave, Sicily. The adult male, top right, is 90 cm high at the withers and the juvenile, back left, is 30 cm high, the smallest known specimen of an elephant. The female (left) has no tusks, unlike any other known female elephant. Isoleucine epimerization ages suggest that this population is considerable older than the more robust *E. mnaidriensis* from Puntali cave in northern Sicily (Belluomini, G. and Bada, J. *Geology* 13, 451, 1985). The authors suggest that successive waves of normal-sized continental elephants invaded Sicily and the subsequent isolation resulted in different degrees of dwarfism (photograph courtesy of University of Rome Palaeontological Museum).

the transformation treatment².

With the demonstration that stable transformation of gramineous species by direct gene transfer is possible, the prospect of genetic engineering of cereal food crops is much closer. In principle, there is no host range limitation because bacterium-mediated introduction is not involved. But systems for the isolation, culture and regeneration of plants from protoplasts are required. The major problem in experiments on cereals is the regeneration of entire transformed plants from protoplasts. In addition, transformation frequencies reported so far for direct gene transfer, for tobacco at least, are much lower than those obtained by cocultivation with *Agrobacterium* (about 0.01 per cent compared with 10 per cent), and there may be multiple copies or other modifications to the directly introduced

DNA⁵. The development of methods to increase transformation frequencies and of techniques to transform totipotent cereal cells must be the next step. In other words, this is now a problem of plant cell biology and tissue culture, rather than a fundamental problem of molecular biology. □

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M.G.K. Jones is a Principal Scientific Officer in the Biochemistry Department of the AFRC Rothamsted Experimental Station, Harpenden Hertfordshire AL5 2QJ, UK.

Palaeoecology

Holocene evolution of lakes

from F. Alayne Street-Perrott

BY USING a well-chosen combination of palaeoecological and isotopic studies, J.-C. Fontes and colleagues have been able to trace the Holocene evolution of three small, closed lake basins on the borders of the Great Western Erg, in northern Sahara. Their paper, published on page 608 of this issue¹, is the latest in a lineage that can be traced back to 1715 when, eleven years after he had identified 'his' comet, Edmund Halley presented to the Royal Society a paper "On the cause of the saltiness of the ocean, and of the several lakes that emit no rivers; with a proposal, by help thereof, to discover the age of the world"². In this short but imaginative essay, he laid the basis for the interpretation of variations in the level of closed lakes (lakes without surface outlets) in terms of changes in climate; he also postulated that such lakes increased in salinity as the inflowing waters were concentrated by evaporation.

The links between climate and lakes were elucidated further by Julian Jackson, an eccentric retired Indian Army colonel, who identified three types of 'affluents' into a lake — direct precipitation, surface runoff and groundwater; and three types of outputs — evaporation, surface outflow and groundwater seepage. He contrasted

the different inputs and outputs in terms of salinity and sediment load, and debated the origin of salt lakes, concluding that some were "formed originally by the universal ocean" whereas others owed their salinity to evaporative concentration.

In the century and a half since Jackson's treatise was published, the study of lake-level fluctuations has become an accepted source of information about past climatic changes³. Variations in the water level of closed lakes can be reconstructed both from direct evidence (historical documents, dated shorelines, buried soils in lake-sediment cores, lakeside archaeological sites) and indirectly, using the assumption that the salinity of closed lakes varies inversely with volume. Since many lacustrine organisms, notably diatoms, charophytes, chrysophytes, ostracodes and molluscs, are sensitive to salinity and alkalinity, fossils are often used to infer past hydrological changes. Supporting evidence can be derived from the analysis of stable isotopes (¹⁸O and ¹³C) in lacustrine carbonates.

When radiocarbon dating became generally available in the early sixties, it was soon apparent that the closed lakes in regions such as the Sahara had experienced similar histories⁴. By 1979, it was clear that lakes and swamps had expanded in many tropical regions north of the Equator between 12,000 and 3,000 yr BP (ref. 6). This wet phase has recently been attributed to an increase in monsoon rains induced by the early Holocene maximum of summer insolation that is predicted by the Milankovitch theory⁷.

The three Saharan basins that form the subject of the study by Fontes *et al.* are dry. The water table lies far below the basin floors, although in the past it may

have intersected the surface. The dates for the basins obtained from the lacustrine period span the range 9,000–3,000 yrs BP. Significant discrepancies, however, emerge when records for individual basins are compared. Fontes *et al.* attribute these differences to local hydrological factors rather than dating problems.

This conclusion confirms fears that the fine details of the Holocene climate record may prove hard to reconstruct from groundwater-fed lakes. Lakes in which groundwater forms a substantial proportion of the water budget tend to suffer from two serious defects as palaeoclimatic indicators. First, their response to climatic fluctuations may be damped, delayed, or perturbed by the sudden opening and closing of underground conduits. Second, lengthy aquifer-residence times may result in the introduction of dead carbon into surface waters, giving rise to anomalously old radiocarbon dates.

Another important finding is that, during phases of low lake levels, the fossil assemblages and isotopic values mimicked marine conditions, although the sites lie more than 500 km inland. The lake deposits contain 'marine' foraminifera, diatoms and molluscs, showing that great caution is needed when interpreting evaporitic sequences in arid areas^{8,9}. One giveaway in this case is that individual beds often display a mixture of freshwater and saltwater organisms. Based on the ¹⁸O results, this situation is thought to have resulted from rapid evaporative concentration of seasonal or intermittent influxes of fresh groundwater, a situation that may have been common in the central and northern Sahara¹⁰.

The initial phase of euphoria about the value of lake-level fluctuations as an indicator of past climatic changes has been replaced by a more cautious and critical mood resulting from the discovery of various complications, notable those associated with groundwater and anthropogenic effects. Nevertheless, the multidisciplinary approach adopted by Fontes *et al.* holds great promise for tackling some of the searching questions about the hydrological and chemical evolution of lakes posed by Jackson over 150 years ago, over a century after Halley's paper. □

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F. Alayne Street-Perrott is in the School of Geography, Mansfield Road, Oxford OX1 3TB, UK.

100 years ago

The third International Congress of Geologists, postponed last year on account of the spread of cholera in southern Europe, has just been held at Berlin. The use of French as the language of discussion was no doubt one effective cause of silence on the part of many members who would otherwise only too readily have made themselves heard. Under such circumstances the Latin races have of course a considerable advantage over the Teutonic.

From *Nature* **32** 599, 22 October 1885.

Electrophysiology leech giant salivary cells

SIR—We note that our description¹ of the electrophysiology of leech giant salivary cells has been echoed by Jones *et al.* in a recent letter to *Nature*². Attention has again been drawn to the possible experimental utility of the cells in studying cellular secretion, but in spite of its title, the letter does not present a study of excitation–secretion coupling. The authors suggest that it should be possible to investigate the release of identified secretions from each of the five types of salivary cell, in the way that serotonin release from the giant cerebral neurones of *Aplysia* has been studied³.

Although the lack of electrical coupling between the salivary cells facilitates study of their membrane properties, a number of problems confront an investigation of the release of identified secretions at the level of the single gland cell. Since no acinar or duct structure is present, it seems likely that the various secretions are formed entirely by exocytosis, without an accompanying volume of solvent. The collection and assay of such small volumes of concentrated product from a single cell will require both the site of discharge and biochemical nature of the secretion to be known while electrical activity is monitored. However, unlike identified neurones in invertebrate ganglia, the histochemical types of salivary cell do not appear to be identifiable either on the basis of stereotyped position and size of their cell bodies within the gland, or by characteristic forms of the action potential. As an alternative to biochemical assay of the product, direct observation of secretory events at the ductule terminal of a single cell is a tantalizing possibility.

Using immunohistochemical methods, we have observed paired nerve fibres with a positive reaction to anti-serotonin antibody originating at the sub-oesophageal ganglion and running along the length of the proboscis sheath to enter the anterior salivary glands at the proboscis base. These axons have multiple branching points and conspicuous varicosities, and

are likely to be the stomatogastric nerves⁴. Although we have not resolved contacts with individual salivary cells, neuronal serotonin is clearly present in their vicinity, and is probably one of the natural stimuli for excitation, as suggested in the recent letter.

The establishment by Sawyer² of a facility for breeding *Haementeria* is greatly to be welcomed, and should allow development of the potential of the salivary gland system, which by now has been very amply stated.

CAMERON G. MARSHALL
Department of Pharmacology,
University College London,
Gower Street,
London WC1E 6BT, UK

CHARLES M. LENT
Division of Biology & Medicine,
Brown University,
Providence,
Rhode Island 02912, USA

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An avian water-repellent proposed

SIR—Bird droppings recently fell onto the roof of my car and overnight there was a very heavy downfall of rain. In the morning, I noticed that although the roof of the car was covered by rain droplets, an area of about nine inches radius around the bird droppings was completely dry.

As the photograph shows, the appearances resemble the inhibitory zone seen on bacterial plates around antibiotic impregnated disks.

It is possible, therefore, that birds have in their bodies a substance with powerful water-repellent properties and that some of the excess of this is excreted in their faeces.

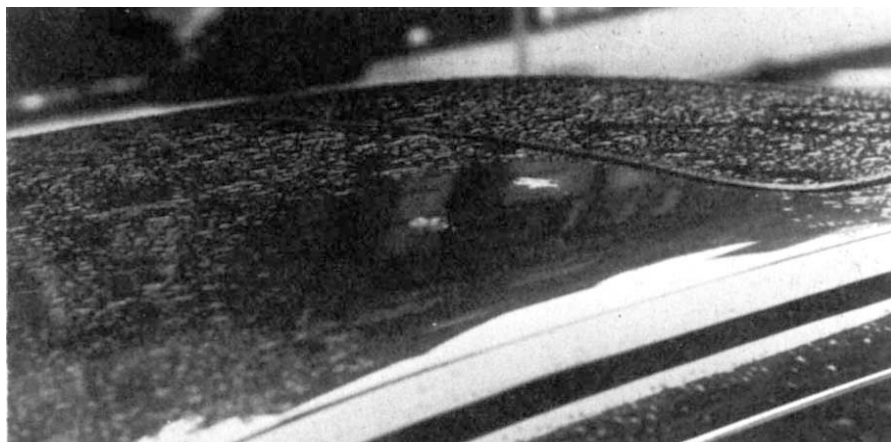
L. ROODYN
Hospital for Tropical Diseases,
4 St Pancras Road,
London NW1 0PE, UK

Symmetry-coded cells in the visual cortex?

SIR—In their comment¹ concerning our recent findings on compound grating discrimination², Livingstone and Hubel argued against the Fourier-analysis theory of cortical function and proposed an explanation of our data in terms of known receptive field properties of cortical cells. We believe that their interpretation is consistent with what we were saying, but that our results are not fully covered by current physiological knowledge. Thus we suggest that it would be useful to investigate whether “symmetry-opponent” cells exist in visual cortex. Such neurones might constitute the missing link in Livingstone and Hubel’s physiological interpretation of our psychophysical observations. Alternatively, new concepts to explain visual pattern analysis need to be considered.

Descriptions of the stimuli themselves do not explain vision. This has been pointed out by Watt³ in his commentary on our study. In this sense we used Fourier terminology for precisely characterizing our stimulus patterns, but we did not imply that a multi-channel model of spatial frequency analysis is adequate for explaining early visual processing in pattern discrimination. Rather, our experimental results led us to conclude that compound grating discrimination can neither be related to narrow-band spatial frequency channels nor to the visual encoding of spatial phase. With these two assumptions being rejected, there was little left to support a global Fourier-analysis theory of compound grating discrimination. This is why we suggested that both non-mirror-image and mirror-image grating pairs are distinguished with respect to specific aspects of grating luminance profiles. Without referring to known receptive field properties of cortical cells, we have also indicated how the two processes of grating discrimination proposed could be related to receptive field sensitivities.

Several observations are supportive of Livingstone and Hubel’s physiological interpretation of our findings. First, when mirror-image grating pairs were compared in extrafoveal vision the darker stripes were as clearly seen as in foveal vision. But discrimination of the pairs was impossible because the darker stripes lacked a precise positional relationship with their background. To the present author as a subject, these stripes appeared floating on the coarser gray striation. Livingstone and Hubel explain why visual resolution (as well as grating contrast sensitivity⁴) may be less reduced in peripheral vision than the processing of positional information. The apparent qualitative difference in handling form foveally and peripherally (our interpretation) could well result from such a difference in rates of deterioration with eccentricity. Second, further support for a re-



A North London car roof betrays water-repelling activity.

when cellular oncogenes are activated by receptive field interpretation comes from a more recent observation on mirror-image grating discrimination — that performance is independent of the number of grating cycles exposed (I.R., M. Hübner and T. Caelli, manuscript in preparation). That is, the underlying mechanism is strictly local. Third, the position of our stimulus patterns varied randomly between presentations. Thus the neural mechanism at issue should have the very property in terms of which Hubel and Wiesel⁵ characterized cortical complex cells: the loss of (*absolute*) spatial localization conveyed by simple cells.

While we agree with Livingstone and Hubel that the difficulty in discriminating mirror-image patterns is related to spatial localization, it is not apparent how a cortical cell of known receptive field properties may distinguish between mirror-image grating bars. A class of symmetry-opponent cells would need to be found to explain how the *relative* position of the darker stripes with respect to the background striation is encoded. Such units are expected to give on- or off-responses to bars with skew luminance profiles (or to edges), depending on whether the luminance peak is on the one or on the other side of the bars. When bars with symmetric luminance profiles were shown, the cells should give little or no response. We feel that the existence of such mechanisms for breaking spatial pattern symmetries would be indispensable for understanding the quality of form at the level of single unit responses. Otherwise, the idea of neural template matching would seem insufficient and relational concepts of visual pattern analysis⁶ should be considered.

It is obvious that symmetry-opponent cells would be functionally analogous to colour-opponent cells. While the first would encode the relative position of peaks (or troughs) within a spatial luminance distribution, the latter recover information on how the spectral energy is distributed within the wavelength domain. Whether or not such an analogy between the visual analysis of form and colour is more than pure speculation has to be established by physiological research.

INGO RENTSCHLER

*Institut für Medizinische Psychologie,
Goethestrasse 31,
D-8000 München 2, FRG*

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The immunology of host-tumour relationships

SIR—In their helpful review of the impact of molecular biology on our understanding of the development of tumours, George and Eva Klein say *inter alia* that

when cellular oncogenes are activated by non-viral mechanisms “the transforming oncogene products are either normal or only slightly modified cellular proteins” and “it is difficult to see how they could provide a rejection target for the immune system”.

Yes, indeed, but they omit to say — and the omission may mislead readers less well informed on these matters than the Kleins — that there may be associated phenotypic changes, and that these may include the expression of what George Klein himself has called “tumour associated antigens with a rejection inducing potential in the autochthonous host (TAARIPAH)”. This, it would seem, is the explanation of the high immunogenicity of many chemically-induced animal tumours. Whether tumours which develop in animals or humans as the result of exposure to environmental carcinogens will also be immunogenic depends partly on the properties of the carcinogen and partly on the pattern of exposure; in general, it seems that a single large dose, such as is commonly used experimentally, is much more likely to induce an immunogenic tumour than chronic exposure to small doses of the same or similar agents, which is what happens in many animals or patients who develop so-called spontaneous tumour.

MICHAEL WOODRUFF

*MRC Clinical and Population
Cytogenetics Unit,
Western General Hospital,
Crewe Road,
Edinburgh, UK*

1. Klein, G. & Klein, E. *Nature* **315**, 190–195 (1985).

KLEIN AND KLEIN REPLY—Woodruff's points are very well taken. We wish we could relate the rejection-inducing antigens of the chemically induced rodent tumours to the oncogene field. The fact is, however, that more than 25 years after their discovery, the nature of these remarkable antigens is still unknown. We find essentially nothing new on the subject since we last reviewed it in 1977 (*Proc. natn. Acad. Sci. U.S.A.* **74**, 2121–2125).

One of the important facts mentioned by Woodruff, the difference in the immunogenicity of tumours induced by strong versus weak carcinogens, probably relates to the latency period prior to tumour development. As shown by Old, Prehn, Baldwin and others, immunogenicity is inversely related to the latency period even within a group of tumours induced by the same carcinogen in the same inbred strain. This is probably due to immunoselection. Highly antigenic tumours can only develop if they outpace the immune response. With the passage of time, low or non-antigenic tumours would have a selective advantage and therefore dominate the picture. As Woodruff points out, the latter is a better model of tumour development after the exposure of humans to environmental carcinogens than

are experiments conducted with strong carcinogens.

Nevertheless, the molecular basis of the highly specific, individually distinct antigenicity of chemically induced rodent tumours is of the greatest interest. In view of our present ignorance, speculations may range from specific interactions of the carcinogen with certain hot spots of the genome, coding for crucially important surface moieties, perhaps related to the major histocompatibility complex (MHC), to phenotypic changes due to activated oncogenes, as Woodruff suggests.

In the latter context, the effect of the mutation-activated products of the *ras* oncogene family are of particular interest. We have previously argued that immune surveillance against potentially neoplastic clones is most efficient in cases where the host species has been preselected to *anticipate* a large number of transformants with the same or similar antigenic changes. This is best exemplified by the virtually watertight surveillance of mice against polyoma induced tumours or of humans against Epstein-Barr virus (EBV) transformed lymphocytes. Although the nature of the target antigens has still not been clarified, it is noteworthy that products of the viral genome like polyoma virus MT or the LT-3 protein of EBV, the most likely candidate targets of the surveillance reaction, are associated with the inside, or the lipid bilayer of the plasma membrane, rather than the outside. They cause changes in the cell membrane that readily evoke a T-cell mediated, rejection geared response, with avoidance of suppression, but do not, or only with great difficulty, induce the formation of serum antibodies.

As Weinberg repeatedly emphasized, tumorigenic activation of the *ras* oncogenes cannot by itself lead to tumour development *in vivo*, since its consequences would be lethal for most members of the species at an early stage. This may not happen because of the multi-step evolution of tumours, as discussed in our review, that is, the need for additional genetic changes. Since the p21 *ras* protein is associated with the inner plasma membrane, it is also conceivable, however, that the host immune system may recognize a mutation-altered *ras* product, particularly if it would influence the expression, presentation, or specificity of MHC products.

In view of the frequent mutations of the *ras* genes, a similar “immunological anticipation” may exist as in the viral tumours. This possibility could be readily explored by transfecting non-immunogenic tumours with appropriate *ras*-carrying constructs.

GEORGE KLEIN
EVA KLEIN

*Department of Tumour Biology,
Karolinska Institutet,
S-104 01 Stockholm,
Sweden*

Down to the foundations

Philip Pearle

Open Questions in Quantum Physics. Edited by G. Tarozzi and A. van der Merwe.
Reidel: 1985. Pp.426. Dfl.160, \$59, £40.75.

Is quantum theory going to provide our final understanding of nature? And, if that is the case, what exactly is it that we will then understand?

In the past decade there has been a resurgence of interest in these questions, stimulated largely by a 1965 paper of John Bell. Yet the current ferment has strong roots in the first third of this century, when every physics conference was on the foundations of quantum theory. The historic fifth Solvay conference in 1927, representing the peak of that activity, solidified the formalism and interpretation of quantum theory. But that conference also saw Einstein criticize quantum theory as an incomplete description of reality, and heard de Broglie present his "pilot-wave" theory, which was to him a more complete description of reality.

While both Einstein's criticism and de Broglie's alternative were and are dismissed by most physicists, these ideas are by no means dead, as the proceedings of this conference, held in the physics department of Bari University in 1983, show. The first four-tenths of the articles are devoted to examining the legacy of Einstein's criticism. A similar number of articles then consider substitutes for quantum theory, which have so far not strayed much beyond de Broglie's original vision. The remainder of the contributions discuss interference experiments, whose inclusion L. Mandel justifies by the remark, "Interference, whether of photons or material particles, tends to violate some of our intuitive notions of physical reality". This, too, is in keeping with tradition, for W. L. Bragg and A. H. Compton spoke on experiments with electromagnetic radiation at the fifth Solvay conference.

From the 1930s until about ten years ago, physicists interested in the foundations of quantum theory were hard put to find conferences to go to and people of like interests to talk with. The overwhelming majority of physicists were, and still are, mining that seemingly inexhaustible lode of phenomena laid bare by quantum theory. However, in the past few years there has been a respectable average of about two conferences a year on foundational questions. The present conference is perhaps typical, both with regard to content and to the wide range of clarity, authority and idiosyncrasy to be found in the 25 invited papers.

The antecedent of this renewed activity was the 1935 paper of Einstein, Podolsky and Rosen (EPR) entitled "Can Quantum-Mechanical Description of

Physical Reality be Considered Complete?". In that paper EPR considered a physical system, describable by quantum theory, consisting of two particles, far removed from each other such that a measurement of the position of, say, the left-particle, permits one to know the position of the right-particle, and likewise for a velocity measurement. They argued that the right-particle cannot be influenced by a left-measurement (whichever measurement, position or velocity) because it is too far away. Therefore the right-particle has to possess both position and velocity simultaneously as "real" properties. But quantum theory is not a description which allots simultaneously a value of position and a value of velocity to a particle — indeed, the Heisenberg uncertainty principle forbids this. Therefore, concluded EPR, quantum theory is incomplete: there ought to be a deeper theory which allows simultaneous position and velocity to a particle in such a situation.

A classical analogue of this is two balls bowled simultaneously in opposite directions, to right and left, with equal speeds from a known location. A person on the left who knows this and who observes the position or velocity of the left-ball can immediately deduce the position or velocity of the right-ball. Of course, in this example the right-ball *does* possess simultaneously both position and velocity, and that was EPR's point: a model such as this *ought* to be constructible.

Bohr's answer to this criticism, that position and velocity cannot be thought of as simultaneously real properties of a particle because they are rather properties of incompatible experimental arrangements, did not convince Einstein and others that they were wrong. It remained for Bell to give a jarring response. Bell considered a mathematically simpler version of the EPR experiment which David Bohm created for his 1951 textbook *Quantum Theory*. Again, there are two particles far removed from each other, but the measurements are of spin projections in different directions and not of position and velocity. Bell showed that, if EPR were right about the existence of a deeper model whose predictions agree with those of quantum theory in this case, then the model must necessarily have the two particles interacting instantaneously with each other, no matter how large the distance between them (this is called "non-locality"). In other words, the completion of quantum theory that Einstein called for

appears to require action-at-a-distance, a violation of Einstein's own relativity theory. No wonder Bell wrote that this was "a resolution Einstein would have liked least".

Bell's result has led to a series of experiments to check whether the predictions of quantum theory are actually fulfilled by nature in this situation. The affirmative answer, most recently given by the experiments of A. Aspect and co-workers, leads one of the contributors to the volume, E. Santos, to declare that

... there are three possibilities: rejecting realism, rejecting locality, or searching for a loophole in the interpretation of the experiments. Here, realism means the belief that material systems have properties that are independent of whether these are measured or not. In my opinion, this is the last thing one should abandon.

Now, Bohr is regarded as having rejected realism. As no spokesman for the Copenhagen interpretation seems to appear at conferences such as this, what are discussed are experiment, locality and anything else that ingenuity suggests may make Bell's result palatable. Some examples: in the experimental realm, T. W. Marshall *et al.* believe they have found some "loopholes" and J. Six believes he has closed some. On the locality front, K. Kraus, in a very lucid paper, criticizes as inconclusive some arguments of H. Stapp and W. Rietdijk that quantum theory itself (not a Bell model) is nonlocal.

As for ingenuity, Bell's result has led to the appearance of a spate of theoretical papers over the past two decades, a flow of different points of view that amazingly shows no sign of slowing, as this book attests. Thus D. Aerts tells us "that quantum mechanics cannot describe separated physical systems and that this incapacity is at the origin of the EPR paradox", and we discover that two particles which are far apart may not be separated, according to Aerts's thoughtful analysis. K. R. Popper declares "... there is no EPR paradox; what is paradoxical is only the Copenhagen interpretation". T. D. Angelidis believes he has shown that Bell's conclusion is unwarranted because there is a much larger class of models than Bell considered. And so it goes on.

The second major theme of this conference is the development of models that in some way "explain" quantum theory. de Broglie's model was abandoned by him because of its discouraging reception at the fifth Solvay conference, but it was rediscovered by Bohm in 1952. In this model, a particle is a decent, classical, point-like object. Bohm showed that the Schrödinger equation may be written as two equations, one being essentially Newton's second law of motion for the particle (the other one describes the conservation of a large number of such particles). However, the force on an individual particle is most peculiar, at least by Newtonian standards,

for it "depends in a nonlocal manner on all the other trajectories which it [the particle] may have taken" (T. W. Marshall, in criticizing Bohm's theory). It is the magnitude of the quantum wavefunction that exerts this force, "piloting" the particle in an erratic trajectory.

Bell's result has provoked renewed interest in this model, because it is an explicit example of the nonlocality deemed necessary to give the predictions of quantum theory. The nonlocal force, which used to be considered a Defect, has become a Virtue. A delightful review of the set of ideas accompanying this model, together with computer pictures of the nonlocal potential and individual particle trajectories in various physical situations, is given by B. J. Hiley, a colleague of Bohm. Among three attempts at relativistic generalizations is P. Gu  ret's replacement of the point particle by an extended object, a soliton, in the spirit of de Broglie's "double-solution" theory, the original motivation for his 1927 pilot-wave model.

Surprising to me is the omission from this collection of an article on E. Nelson's 1966 stochastic model, which I regard as being at least as interesting as the de Broglie-Bohm model. By considering that a particle undergoes a peculiar kind of time-symmetric random walk, Nelson is able to arrive at the Schr  dinger equation. Included, however, is an article by E. Santos on stochastic electrodynamics which starts with a purely classical picture of a particle immersed in a randomly fluctuating electromagnetic environment, and then attempts (unsuccessfully, so far) to drag the Schr  dinger equation out of it.

The last few papers in the book deal with experiment. Two of them discuss ways one might detect a de Broglie-Bohm "empty" pilot-wavepacket containing no particle, A. Garuccio's being quite brief.

I have saved for last the three articles which are based solely on quantum theory. M. Cini presents a beautiful model of a quantum measurement. L. Mandel gives a brief but lucid description and explanation of optical interference experiments, including interference of two separate lasers. And H. Rauch carefully discusses some of the amazing neutron interference experiments which "... provide a unique tool for the realization of quantum mechanical textbook experiments on a macroscopic scale ...". The authority of such work, based upon the solidly established formalism of quantum theory, when compared to the questing nature of the rest of the articles, recalls the prognostication of Einstein that, although the "statistical quantum theory" (as he insisted on calling it) would eventually be replaced by a more complete theory, "... the path will be lengthy and difficult". □

Philip Pearle is Chairman of the Department of Physics, Hamilton College, Clinton, New York 13323, USA.

Heavy-weight renal regulations

J.F. Lamb

The Kidney: Physiology and Pathophysiology, Vols 1 and 2. Edited by Donald W. Seldin and Gerhard Giebisch. Raven: 1985. Pp. 2,273. \$289.50.

THESE two books are a comprehensive account of the current state of renal physiology, "conceived broadly as the study of those processes by which the kidney maintains the volume and composition of the body in the face of physiologic demands and pathologic disturbances".

The text is organized into four sections, the first of which deals with the general principles of electrolyte regulation, with main divisions into the organization of the body fluid compartments and solute-solvent transport. Section II is on the organization of the kidney, both structural and functional, and includes (perhaps rather perversely, for regulation in the kidney is in the next section) description of the renal regulation of certain extrarenal functions such as blood pressure. Section III gives a detailed account of the exchanges and regulation of water and the common electrolytes such as sodium and chloride, potassium, and so on, the trace elements, proteins and other macromolecules, both in normal and abnormal states. Section IV deals with renal failure and pharmacology.

Most of the chapters are quite short with a welcome use of explanatory diagrams and straightforward description of the problems in that particular area. Many have a fairly detailed, though not usually overlong, account of the history of the subject concerned; this does help to introduce it and puts present views into perspective. For example, it is pleasant to see the book start with Walser's model for the overall control of electrolytes and water excretion. Although this topic is discussed in mathematical terms, it is based on the

idea of considering the body as a bucket with a hole in its side: the fluid level at the hole represents the zero point at which renal excretion is negligible, while levels above this drive excretion proportionally; inputs enter through the open top of the bucket. This simple model seems to fit the available data better than others and should be useful as a "mental picture" in designing tests of the process and for teaching. The succeeding chapters make equally rewarding reading, both for specialists and for the generalist.

Books such as these are a compromise between allowing experts the freedom to write endlessly on their pet subjects and keeping the length and complexity of the final text to a reasonable size. The editors have, I think, struck a reasonable balance between these conflicting requirements. This has been achieved, partly at least, by careful selection of the contributors who seem to have been chosen both for their ability to communicate the material as well as for their knowledge. The standard of production is high, with clear illustrations and (as far as I could see) few mistakes. The authors provide adequate cross-references to other sections of the books, and there is not much obvious duplication of material (though I did notice two near-identical tables—No. 2 on p.366 and No. 4 on p.415). Such extensive coverage of the subject has some penalties, mainly in the weight of each volume (over 8lb); for the not inconsiderable price, the publishers might have included a pair of book rests.

Overall, this is an excellent work, well deserving a place in departmental libraries where it will be consulted for information not only on the kidney but also on transport processes, current views on blood pressure regulation and much more besides. It should suit more senior students, postgraduates and research workers who wish to keep abreast of developments in these areas. □

J.F. Lamb is Professor in the Department of Physiology and Pharmacology, University of St Andrews, Bute Medical Buildings, St Andrews, Fife KY16 9TS, UK.

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Female humpback whale with calf. The picture is taken from *Wings in the Sea: The Humpback Whale* by Lois King Winn and Howard E. Winn. Publisher is University Press of New England, price is hbk \$25, £23; pbk \$15.95, £15.30.

On the written authority of Ernst Mayr

Ernst Mayr's The Growth of Biological Thought: Diversity, Evolution, and Inheritance was first published in 1982 to widespread acclaim. Three years later, on the appearance of the paperback edition, W.F. Bynum reassesses the book.*

BIG books, especially if written by Harvard professors, often pack authority between their oversized spines. *The Growth of Biological Thought* was not the first heavy tome written by Ernst Mayr, nor the last he promises. His *Systematics and the Origin of Species* appeared more than 40 years ago, and the present volume is the first of two projected ones on the history of Mayr's favourite subject, biology. It deals with what Mayr calls "ultimate" biology, essentially the causes and consequences of evolutionary processes. The companion volume will chart the biology of "proximate" (functional) causations, that is physiology, embryology and neurobiology.

Appropriately enough, the hardback edition of *The Growth of Biological Thought* appeared in 1982, the centenary of Charles Darwin's death. It was a publishing triumph, well-produced, reasonably priced and widely reviewed, not simply in scientific journals but in several newspapers (*New York Times*, *Washington Post*), literary publications (*New York Review of Books*) and general high-brow magazines (*The New Yorker*). Steady paperback sales are undoubtedly anticipated and it will probably remain in print for years. Although it now has a substantial rival in Peter Bowler's *Evolution: The History of an Idea*, published by the University of California Press in 1984, it can be predicted that, for the foreseeable future, Mayr's book will be cited as the definitive history of evolutionary thought. "This is an extraordinary epic work in which Mayr once again shows himself a master of detail, interpretation, and synthesis", wrote the reviewer in *Science*. Others described it as solid, vast, awe-inspiring, breathtaking, a crowning achievement, an encyclopaedic tour de force, a great book.

We can agree that it richly deserves all these epithets and yet still find in the volume (as pointed out in a minority of less favourable reviews) other, two-edged features. Before looking at these, let me describe for those who have not yet read this thousand-page opus, what to expect. A short prolegomenon justifies Mayr's decision to write an intellectual ("problems, not periods") rather than a social, cultural, chronological or biographical history of his subject. This short justification is buttressed by a longer lament that most philosophers of science have based their ideas and models of science on physics and reductionism and have been ignorant of the important ways in which organismal

biology has its own special problems and methods. For Mayr, "teleology", shorn of its metaphysical implications, is not something which modern life science has outgrown, but actually central to ultimate causation, that is, evolutionary processes. This preliminary survey allows him to develop briefly his two main, recurrent themes: the contrast and tensions between essentialist (or typological) and population thinking, on the one hand, and



between "soft" (Lamarckian) and "hard" (Weismannian) inheritance, on the other.

Three key phenomena make up ultimate biology: diversity, evolution and inheritance, and these are treated at great length in turn. The most original section is that on diversity, where Mayr puts his own vast experience of the problems and philosophy of systematics to good use in surveying the history of biological classification and taxonomics (both macro- and micro-) from Aristotle to the 1980s. Darwin of course occupies a pivotal position in this section and is centre-stage in Part II, which examines the inevitable cast of "forerunners" (with a reasonably sympathetic exposition of Lamarck's achievements) and the fate of natural selection up to and beyond the "evolutionary synthesis" of the 1930s and 1940s, in the construction of which Mayr himself played such an important role. Part III picks up the other thread of the synthesis, inheritance, from the traditional theories of soft inheritance (of acquired characteristics) through Mendel, Weismann, de Vries, Morgan and up to DNA. A disarming epilogue speculates about the possibility of a "science of science". Those daunted by the book's size but wanting a fuller description of its contents may be referred to A.J. Cain's review in this journal (*Nature* 297, 707-709; 1982), to George Gaylord Simpson's discursively revealing com-

ments (*The Quarterly Review of Biology* 51, 437-444; 1982) or to John Maynard Smith's sympathetically critical exposition (*New York Review of Books* 41-42, 13 May, 1982).

I have in addition read more than a dozen other reviews of the original edition, virtually all of them by practising biologists. From this sample I brought away two observations and had three conclusions confirmed about Mayr's monumental tome. The first observation is that the best scientists are the best writers (in which category the three reviewers mentioned above belong). "*Le style est l'homme même*", remarked another great biologist, Georges Buffon. Mayr himself offers a few brief reflections (pp.838-839) on the relationship between the form of publication and the chances of getting anyone to notice. Someone should do a more systematic study.

The second observation, more substantial perhaps, is the role of authority in a book's reception. Mayr has remarked, "If I have been successful at all, it is because I have myself done a considerable amount of research in most areas covered by this volume". His reviewers for the most part agree: one of the towering figures of evolutionary biology has now written a towering history of his discipline, is the general drift. Mayr's familiarity with and understanding of the biological phenomena he has dissected historically are everywhere apparent, as are his omnivorous reading and superb expository skills. That same authority stamps his personality on every page of the book. Consequently, *The Growth of Biological Thought* possesses three closely-related characteristics which somewhat detract from its overall historical value.

First, it is Whiggish, despite Mayr's claims to the contrary. Whig history constructs the past as a series of steps leading to the present and awards points to past figures who "got it right", or who thought most nearly like we do today. Much history of science smuggles in a little Whiggism, but most historians of science now attempt to evaluate the past on its own terms. Mayr insists that he pays "special attention to underdogs" (both persons and theories), but all too often he fails to reflect the importance his characters had in their own times. Thus, Erasmus Darwin, Richard Owen and Herbert Spencer are relegated to the sidelines, while Robert Chambers (although he gets four pages) is dismissed as "an ignorant layperson".

Secondly, Mayr consistently bewails the role of Christianity in biological thinking, viewing the relationship between science and theology with the traditional (but now historically discarded) metaphor of warfare. "I have analyzed . . . the set of ideas and beliefs that had prevented an earlier acceptance of evolution. It consisted of natural theology and a very literal creationism together with essentialism".

*Published by Harvard University Press, pp.974, \$12.95, £10.95.

The prevalence of contemporary fundamentalism may have coloured Mayr's attitudes, but they rob him of a good deal of historical sympathy and cause him to undervalue a lot of historical scholarship on the subtle interplay between cultural beliefs and scientific discoveries. Mayr himself seems uncertain about this issue, for while granting the "power of ideologies" in scientific thinking (pp.834-835), he elsewhere questions whether any connections exist "between the scientific advances and the general intellectual and social milieu" (p.124).

What is certain, however, are the connections between Mayr's scientific beliefs and the kind of history he writes. He is suspicious of mathematics in biology, and has little time for biometricians or the more recent computer-based taxonomists. He dislikes contemporary theories of macroevolution, so his former student Stephen Jay Gould is conspicuously absent from his long list of acknowledgments and virtually absent from his text. In fact, it could be argued that Mayr's book is not history in the conventional sense, but what Simpson's review playfully called "autobiology" and what John Henry Newman, custodian of a faith for which Mayr has no time, might have dubbed an *Apologia pro vita sua*. □

W.F. Bynum is at the Wellcome Institute for the History of Medicine, 183 Euston Road, London NW1 2BP, UK.

Odd astronomers and other curiosities

David W. Hughes

The Astronomical Scrapbook: Skywatchers, Pioneers, and Seekers in Astronomy. By Joseph Ashbrook. Cambridge University Press/Sky Publishing: 1985. Pp.468. £12.95, \$19.95.

SCRAPBOOKS, stamped with the collectors' idiosyncracies, can hardly fail to be fascinating. From the very meaning of *Scrapbook* in the title of a book, you know that you are going to be led down a series of byways, that the items will be easily digestible, and that you can happily dive in at random, enjoy an intellectual snack, and then put the collection aside for a future treat. And you know that you will be taken by surprise.

Joseph Ashbrook (1918-1980) joined the staff of *Sky and Telescope*, America's foremost astronomical magazine, in 1953. Every other month for 23 years he wrote a column entitled "Astronomical Scrapbook". He was intrigued by the human aspect of astronomy and revelled in accumulating details about obscure, overlooked astronomers whose fleeting fame had long since been forgotten. He constantly reminded his readers that the history of astronomy is not a tale of steady, relentless progress, but one that reflects life, where mistakes, failures and frailty are all too common.

Ninety-one of Ashbrook's best articles have been assembled into this book, sub-

divided into seven sections: "Star Crossed Lives" (a collection of biographical essays); "Telescopes and Techniques"; "Phenomena of the Earth, Moon and Planets"; "Studies and Students of the Moon"; "Planets and other Solar System Objects"; "Stars and Stellar Systems"; and, finally, "Star Atlases".

We read of August Sonntag ("a tantalizing figure, dimly glimpsed") and Smyth's *Celestial Cycle* ("a cloudy-night solace to the amateur observer"); of immense telescopes which were "only about as useful as the enormous spectacles which were suspended over the doors of opticians"; and of Dr Peate, a clergyman who figured mirrors, none of which ever seemed to have been put to serious use ("by presenting them to colleges that could not find funds to build and staff observatories, he effectively buried them"). What is an Angosiade? What did Dawes' dog Dash do? How long does it take to estimate the colour of a star? Answers to all these questions are here, and to many more besides.

A typical Ashbrook line is "The story involves an Austrian Naval officer, a Massachusetts clergyman, a German school teacher, and a group of mathematical astronomers at the University of Cincinnati". But underlying each story line is Ashbrook's scholarship, exactitude and love of detail. The book kindles a desire to learn more, a desire which can be satiated thanks to an extensive reference section. This is a gem of an anthology which can be savoured time and again. □

David W. Hughes is Senior Lecturer in Astronomy and Physics in the Department of Physics, University of Sheffield, Sheffield S3 7RH, UK.

Pictures of the past

W.S. McKerrow

Elsevier's Invertebrate Fossils Chart. By P. Lof. Elsevier:1985. 10 copies Dfl.235, \$87, with reduced rates for larger orders.

ELSEVIER's latest chart, for which you first require a large (95 × 135cm), clear space of wall, shows coloured photographs of over 150 fossils, arranged taxonomically. There are notes explaining the classification of the common invertebrate phyla, their classes and orders, and annotated diagrams illustrating some of the more usual morphological terms. Smaller graphs indicate the stratigraphic ranges of the commoner classes and orders.

The photographs are taken mostly from A.E. Richter's book, *Fossilien zum Sameln*, published by Franckh'sche Verlaghandlung in 1980. The fossils are predominantly from Germany, France, Bohemia and Scandinavia, with 20 or so from the United States and a sprinkling from other countries.

The chart will form a pleasing decoration to a student's study, but not everyone will find the price as attractive; several introductory textbooks cost only about double the amount and provide much fuller descriptions and illustrations. Moreover, a real beginner may be confused by so many names of orders, and will certainly find much of the terminology perplexing. Despite the claim that the chart will provide students of geology with all the information they need about invertebrate fossils, I would still recommend a good introductory text which indicates fully how and where these animals lived, and which uses a minimum of technical terms. For myself, I will continue to prefer to use real fossils as examples in teaching.

Still, the chart is useful ancillary material, and if a wall chart is what you want this one is clear, accurate and colourful. To some extent, the photographs speak for themselves and will certainly help the student and the amateur collector to recognize examples of the major groups of invertebrate fossils. □

W.S. McKerrow is a Lecturer in the Department of Earth Sciences, University of Oxford, Parks Road, Oxford OX1 3PR, UK.

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Anti-darwinian theory in Japan

from Beverly Halstead

The popularity of Kinji Imanishi's writings in Japan gives an interesting insight into Japanese society.

KINJI Imanishi (born 1902), Emeritus Professor of Kyoto University, is a noted figure in contemporary Japan, a renowned mountaineer and explorer, and the recent recipient of Japan's Order of Culture First class. In his earlier days, he was a student of mayflies and habitat segregation¹ and the behaviour of non-human primates and biosociology². But he is now best known for his numerous books in which he advances his own anti-darwinian view of evolution³⁻⁵.

Imanishi's books are all exceedingly popular and all of his works on evolution remain in print, right back to that which he first had published in 1941. Indeed, it would not be unreasonable to say that in Japan the average intelligent layman's understanding of evolution stems in great measure from the writings and innumerable interviews given by Imanishi.

Imanishi is not simply a popular writer. In Japan he is often held up as an intellectual giant equivalent to Charles Darwin. There is talk of a movement, led by Seigen Tanaka, to recommend Imanishi for the Nobel prize. A new centre of "Japanology" which the Prime Minister, Yasuhiro Nakasone, is pushing hard to set up and whose aim will be to "create outspoken Japanese capable of explaining, defending and exporting their cultural values around the world just as the West has done" will, according to newspapers, have Imanishi as one of its chief advisors.

Imanishi's views certainly do represent different cultural values. He sees the group and not the individual as of fundamental importance in evolution, he does not see struggle in nature but rather the underlying harmony. Imanishi claims that his insights arise out of a particularly Japanese world view, which is starkly at variance with the philosophies and religions of the Western world. Imanishi seems to be dismissive and at times contemptuous of the West and in particular of its individualism.

What Imanishi has to say is of considerable interest. The claim that there was a new Japanese view of evolution, stemming from the intrinsic qualities of Japanese society is especially intriguing.

"On one fundamental point, Imanishi is in complete agreement with Darwin. He accepts that from the origin of the first living things on Earth, life has diversified through the aeons of geological time. Where he parts company with Darwin, is on the importance that Darwin assigns to

the role of the individual, especially with regard to the concept of natural selection, involving, as it does, competition among individuals in the struggle for existence. Imanishi contends there is no such thing as survival of the fittest; what determines survival is in most cases purely accidental. It is a matter of luck rather than selection. Although naturally, Imanishi recognizes that individuals vary, he considers that to a very great extent the nature of the species as a whole determines the character of the individuals. It is not the other way around, as Darwin would insist. Imanishi relates this to the sense of identity that organisms possess. Imanishi sees the species or more properly his species-society as being an entity in its own right.

Imanishi considers that Darwin's view of nature is too harsh: it is a paternalistic view derived from the cultural and religious ethos of Western Europe. The Japanese view is essentially a maternal one, that sees the maintenance of stability as a primary factor. Imanishi stresses that in the development of the individual, from the egg, there is no struggle for survival among the cells, just as there is no competition or struggle for survival within a cell. What one finds as the cells divide and differentiate is change that proceeds smoothly.

The emphasis on the individual and indeed on individualism is a feature of Western society^{6,7}. He writes⁸:

One of the decisive differences between Darwin's theory of evolution and mine, as I mentioned before, is that, my theory holds [that] all the individuals of a species change at once when the time to change comes, Darwin thought that evolution begins with the individual, or with a small number of individuals. This certain individual, using a term of modern neo-darwinism, is called a mutation. Whether in morphology or behaviour, this mutation can be victorious in competition only when it is the fittest, and it is further supposed that that organism losing in the competition for survival will perish. God is always on the side of the elite. Perhaps because this appeals to those Christian Westerners. . .

Some years have passed since I first said that evolution is change occurring when it is time to change. And as support for this assertion. I have produced on many an occasion the example of ontogeny, that is, the growth of the individual. The individual does not change through the process of growth.

Evolution is changing when it is time to change, I said, and this is not just some incidental notion, but my evolutionary theory,

but Westerners will probably not agree with me. They know only the individual, and probably will not recognize anything above the level of the individual.

But this is only one part of Imanishi's evolution theory. Arising out of his early research on mayflies, he developed the concept of habitat segregation, which he later amplified as life-style partitioning. It can be observed that in freshwater streams and rivers one can recognize that species appear to be segregated into particular habitats. This concept is central to Imanishi's evolution theory. He claims that not only does competition among individuals of a species not occur, but neither does competition between species. In this sense, Imanishi's evolution theory is novel and departs from the many others, which reject the notion of *intraspecific* competition. They, nevertheless, are happy to accept *interspecific* competition. Imanishi bases his revolutionary notion on his contention that species, through mutual identification, simply of their own accord, select their particular habitats. In fact, the concept of identification transcends that of adaptation. It is the nature of the species or "species-society" that has primacy over the individual in this matter⁶. The individual species choose their own habitats within the environment. As Imanishi states⁸:

In my younger years I challenged the succession theory of the ecologists Clements and Shelford with the habitat segregation theory, and now I am doing the same, this time challenging Darwin's theory of evolution. This is because both the succession theory of Clements *et al.*, and Darwin's theory of evolution are predicated upon the principle of competition.

The principle of habitat segregation is not a principle of competition, but is most emphatically a principle of coexistence.

I regard the biological nature we see not as the scene of survival competition, but as the scene of peaceful coexistence among species. Therefore I have also said that evolution to me is an increase in the density of the habitat segregation of the species.

But no matter which way one looks at it, I find it quite impossible to reconcile the struggle for survival with the evolution of the harmonized holospecies.

Imanishi draws attention to the fact that the selectionism of Darwin has its roots in Western society, whereas the theory of Imanishi has its roots in Japanese society. The emphasis on the group rather than the individual, the social structure of Japan,



Subject and author

indeed the manner of perception of the world and man and the orderly formation of harmony in society is clearly reflected in Imanishi's theories. In any event, Imanishi contends that there are two quite different and contrasting theories of evolution: that of Darwin and that of Imanishi. Imanishi is strongly critical of neodarwinism with the individual as the fundamental unit of the process of evolution, and attributes the emphasis on competition and selection to the social ambience of the West. To Imanishi, "Darwin got carried away with competition and selection, whereas [Imanishi] chose habitat segregation" and an evolutionary theory based on the principle of cooperation and mutualism — the principle of cooperation and an inherent harmony in the living world.

These two evolutionary theories illustrate his very concept of "habitat segregation": Darwin's inhabits the West and Imanishi's the East."

One of Imanishi's articles has recently been translated into English "A proposal of shizengaku: the conclusion to my study of evolutionary theory". This is difficult to follow without some knowledge of the background and it is not easy to communicate Imanishi's wonderful command of the Japanese language which makes his works so pleasant to read, if not necessarily easy to understand.

The notion of the primacy of the group falls on fertile ground in modern industrial Japan and in a sense the importance of the corporate nature of mankind, which Imanishi emphasizes, does provide a kind of intellectual respectability for the suppression of individualism. Curiously Imanishi's views are popular among left-wing students by virtue of his emphasis on cooperation and mutual aid. There are indeed areas where Petr Kropotkin's *Mutual Aid*, with its emphasis on cooperation, are mirrored in the writings of Imanishi.

The influence of Imanishi is particularly powerful because it encompasses both ends of the political spectrum, no mean feat in this day and age. But there is something more than this, because Imanishi is especially popular with the ordinary Japanese. The fact is that the prevailing concept of competition is detested by the ordinary Japanese, because everyone is involved in it in their real lives. Imanishi

presents them with an alternative — a dreamscape of irrational sentimentality. Imanishi satisfies the dreams and aspirations of people, his writing is comforting and the style is persuasive especially to those with no real background in biology and science. His style is very colloquial and sometimes even in Kyoto dialect. Real life in Japan is very hard for ordinary people, everyone is in a desperate race. Imanishi gives dreams and hope to the Japanese people. Just as between the wars, when foreign travel was not possible, Imanishi and his friends went on mountaineering and exploration expeditions, and made films and books of their exploits. Their activities were envied by the Japanese people, for whom they provided dreams and hope throughout the years of depression and militarism. One suspects Imanishi is fulfilling a not dissimilar role today.

Imanishi's evolution theory is a poetic vision, it is beautiful to contemplate but it is a dream and it is Japanese in its unreality. In the world and indeed in Japan, it is demonstrably a world of competition, struggle and disharmony. In such a world Imanishi's theory acts as a wonderful counterbalance that gives people hope and something towards which to strive. Unhappily it has no place in the scientific understanding of the real world.

One of the most interesting features of the Imanishi phenomenon is that he epitomizes the generation that developed between the wars and was isolated from the mainstreams of international science. It was in just such intellectual isolation that new ideas and concepts were evolved. Before the First World War there was a tremendous influence of the West from the time of the Meiji Restoration (1868) until the rise of militarism. After the Second World War Japan again came to enter the mainstream of the West, at least substantial contacts were effected. The intervening period was one of particular originality from within the Japanese intelligentsia, especially the Kyoto Elite of whom Imanishi stands as a prime example.

Imanishi stood, and stands, firmly against the crude selectionism that was particularly enamoured of the rulers of Japan at the end of the last century and the early decades of the present one. Social darwinism was held to be an adequate jus-

tification for the running of society by a powerful authoritarian government.

The fundamental divide in man's view of the evolution of life, which has fuelled the most vitriolic of controversies, has been the degree of emphasis placed on either observed variations among individuals or on the features held in common. Was individual competition among individuals the driving force of evolution or was it cooperation? It must surely be evident that both aspects must be represented and over recent times there have been attempts to produce a synthesis — a dialectic resolution of the unity of opposites. This is perhaps best exemplified by in the writings of John Maynard Smith¹⁰. It can be fairly confidently stated that mutual aid and cooperation, indeed the phenomenon of altruism, are susceptible to an explanation in terms of natural selection. The antithesis emphasized by Imanishi is a false dichotomy. It is now resolved. There is no longer any foundation for Imanishi's attack on what he imagined darwinism to comprise — it was merely a caricature of Darwin.

So much for Imanishi's views on the inherently harmonious nature of the living world. There are nevertheless a number of empirical claims derived from his early work on habitat segregation on aquatic insect larvae, mayflies in particular, that are critical and form the keystone to the edifice of Imanishi's evolutionary theory.

First is Imanishi's claim that the larvae select their habitat on the basis of recognition of species identity, that there is an innate property of proto-identity and that this determines the association of individuals of the same species. The second major contention is that where two species separate in a similar environment, they separate not by direct competition as usually assumed but simply by one group moving away of its own volition, that direct confrontations are always avoided in the living world.

The association of larvae of the same species is now established to be not a consequence of some type of proto-identity but simply the preference for particular physical parameters of the substrate¹¹⁻¹⁵. Recent researches in freshwater, marine and terrestrial habitats in experimental ecology, have demonstrated that interspecific competition takes place in some 90 per cent of all cases studied¹⁶.

The informational foundation of Imanishi's theory no longer stands, but this seems to have little effect on either the standing or on the public reception of his ideas. For an explanation of this, one has to turn to the scientific community in Japan, which has its own special characteristics. It is characterized by the virtual absence of debate or any kind of intellectual confrontation. The great teacher or leader, the *le-moto*, a member of this elite is untouchable. There are many examples of this situation within Japanese science, which have been incisively dealt with by

Sibatani, who in 1981 wrote a critique of Imanishi's evolution theory¹⁷ but who subsequently rather spoils the effect by being converted to Imanishi's ideas^{4,18}. Perhaps the most striking example comes in the writings of Shoji Ijiri, a Marxist palaeontologist who has taken a special interest in evolutionary theory, but has never discussed the contrary views held by Imanishi. The reason according to Ijiri was that he believed the theoretical concepts were simply "saloon theories from the Kyoto University coffee houses. They were group discussions for aristocrats, who talk about science with their brains and their mouths, in contrast to Ijiri who approaches theory on the basis of practical experience of mud and sweat"¹⁹. As far as Ijiri was concerned "farmers and aristocrats have no points in common"¹¹, in short he had completely ignored Imanishi's theory.

Whichever stand is taken the result is the same, there is no public response. There is one further factor, which may be significant. Imanishi states he is not an ecologist, hence ecologists cease to discuss his ecological theories even though his ideas may be central to their own researches. When Imanishi now states he is not a scientist, fellow scientists no longer feel there is any need to discuss his theories,

even though they are concerned with the central concepts of their own disciplines. There seems to be a rigid regimentation in the natural sciences and great store is taken of labels that are paraded and rather less consideration of the actual content being displayed. There is a handful of exceptions. Sibatani has conducted a series of criticisms of the state of Japanese science³⁰, and the natural sciences in particular, from his working home of Australia (although he is now in retirement and settled in Japan again).

Vigorous debate is not part of the cultural tradition in Japan although deep and learned disagreements and discussions are conducted within groupings such as the Kyoto Elite. Dedication to the search for truth will allow discussion among elites. There is the paradox of the elite proclaiming the importance of the group and Imanishi is a classic case in point, and yet his entire life has revealed the attitude of an extreme individualist. Indeed members of the Kyoto Elite are bound together by a single trait, extreme individualism, but who to a man proclaim the primacy of the group *for the rest of Japanese society*. It certainly does not apply to themselves. Originality and innovation flourish in a secret enclave beyond the experience of

the ordinary Japanese, condemned, as they are, to the rigid authoritarian feudal society that masquerades as one of the advanced nations of the world.

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Beverly Halstead is in the Department of Geology, University of Reading, Whiteknights, Reading RG6 2AB, UK.

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To the defence of economics

from Partha Dasgupta and Frank Hahn

An attack upon the discipline of economics, from within, is repulsed.

A.S. EICHNER's article, "The lack of progress in economics" (*Nature* 7 February 1985)¹ is a remarkable exercise in imaginative writing. He has constructed in his mind a theory of what present-day economists are engaged in when they do economics — what Eichner calls "the prevailing practice among economists" — and proceeds to flail against the enterprise because, in Eichner's theory the current practice "... is based entirely on axiomatic reasoning, without empirical support for the key propositions upon which the theory rests". Economists reading Eichner's article will have noted at once that

his own theory of what other economists do is deeply flawed on this count. But we have no reason for thinking that the majority of *Nature's* readership, who we take it are non-economists, will recognize this, and in particular, recognize the fact that Eichner's claims are simply false from start to finish. Economics, like other disciplines we imagine, has its share of people who, for whatever reason, cannot grasp what they criticize, and ordinarily we would not respond to such an article as Eichner's. In any case, there is no obvious harm in having some dottiness around. But this piece may cause mischief, for

Eichner ends by suggesting that, "... it may ... require the censure, or at least a strong protest, of the scientific community to force a change in the prevailing practice among economists". Since he has carried his frustrations to a multi-discipline journal such as *Nature* it would be improper of economists to let the piece pass by without comment.

We will not engage in a discussion of whether it is possible for economics to become a *science* — the opening summary of Eichner's article — as it is not at all clear what would be meant by a claim that it is not. More to the point, it would have no

bearing either way on Eichner's thesis about the intellectual concerns of present-day economists. Eichner begins by claiming "the core of economic theory consists of four theoretical constructs which serve as the micro foundation for the dominant paradigm . . . indifference curves, isoquants, positively sloped supply curves and marginal physical product of capital curves". Moreover, in Eichner's view "these four theoretical constructs are derived from two fundamental relationships", namely " . . . a utility function representing the utility, or satisfaction, derived from the use of a given assortment of goods . . ." and " . . . a production function representing the different quantities of inputs required to produce the output (*sic*) of the economic system". One of these inputs, so Eichner claims the dominant paradigm requires, is an aggregate measure called "capital", a concept that, in the words of a J. Blatt from whose writings an extract is reprinted alongside Eichner's piece, is impossible to define consistently.

It is difficult to know where to begin. For the economist reader, to quote is to expose. For others this would not be so. Therefore we begin by noting that "the dominant paradigm" in economics, as Eichner calls it, does *not* require and does *not* rely on marginal physical product of (aggregate) capital curves, as a perusal of any leading graduate textbook^{2,3} on microeconomic theory would testify, and it is astonishing that Eichner is unaware of this. Nor does it require the third of Eichner's four putative constructs, namely positively sloped supply curves (see for example refs 2 and 3 or the less technical specialized survey by Killingsworth⁴, in which the theoretical reasoning behind and the empirical evidence for labour supply schedules that are in part *negatively* sloped are discussed). Indeed, the dominant paradigm recognizes very much that a modern economy is composed, among other things, of a complex set of interdependent markets, so that the supply forthcoming of a commodity depends on current prices, expected future prices of various goods and services, incomes of various categories, and so forth.

We have some difficulty understanding what precisely Eichner finds objectionable in the idea that firms can, in principle, choose from a variety of techniques to produce a given commodity (the second of Eichner's four constructs). There is now an enormous empirical literature showing that firms do react to altered economic conditions by saving on this resource and relying more on that resource (see for example Berndt and Field⁵ for evidence of substantial substitution occasioned by oil price rises). Eichner seems to think there is something phoney about such substitution possibilities among inputs in production (but think of double-glazing as a means of lowering the use of oil and gas, to take literally a homely example), and he

seems to suggest (at the bottom of p.427) that there *are* no substitution possibilities among inputs, that there is only one technique — or only a few techniques — for producing a commodity. The dominant paradigm is obviously capable of absorbing such a specification of production possibilities, for it is a *special case* of what Eichner finds otiose.

We have left for last Eichner's most astonishing claim (and that of J. Blatt, whose views are published alongside Eichner's piece), that the theory of consumer choice is founded on the hypothesis that a person's "utility" or "level of satisfaction" can be measured, a hypothesis for which there is no empirical evidence. It is now over fifty years since Sir John Hicks and the late Sir Roy Allen developed empirically refutable propositions regarding consumer demand for goods and services, based on a framework which firmly avoided any such concept as "cardinal utility", or "satisfaction"⁶, a framework which formed a cornerstone of two classics in the literature of economics^{7,8} and is, if one cares to look for it, to be found in any graduate text on microeconomics. Moreover, the empirical work on consumer demand for various categories of goods and services is simply enormous (see for example Deaton and Muellbauer⁹ for a survey and references), and it is incomprehensible to us how Eichner, who we understand has a teaching post at Rutgers, the State University of New Jersey, can have missed all of it. The claim that empirical investigations of the implications of microeconomics theorizing are non-existent is particularly ironic given the extraordinary outburst of publications in the field of applied microeconomics since the Second World War.

How can such remarkable misconceptions be held by a professional of his own discipline? Part of the answer must be simple incomprehension. During the past few decades the subject has developed at a furious rate, largely because it has increasingly attracted talented and well-trained young people, some of the highest ability. The spread in the technical competence amongst economists is very large; by its nature economics is a very technical discipline. It has always been so, and if it did not *appear* to be so it is because earlier economists had adopted a diplomatic style of writing and lulled the reader often into thinking that the analyses were easy. Reading Adam Smith, or David Ricardo, or Karl Marx or Alfred Marshall or John Maynard Keynes is in fact no easy matter. They were grappling with some of the hardest of intellectual problems and were trying to illuminate by abstracting here and simplifying there. For them the matter was harder in addition because they restricted themselves to developing their arguments in spoken language (we will not say *plain* language!) when what they were engaged in was often mathematical reasoning. (The drawback of this

approach to communicating with one's readers can be seen from the fact that nearly fifty years after the publication of Keynes' *General Theory* economists are still debating what Keynes really meant.) Today, such inhibitions have disappeared and a greater part of the most interesting advances in theoretical and applied economics are being made by people who write for each other and who get down to business at once, not indulging much by way of preliminaries about the motivation behind their investigations, preliminary chit-chat and so forth. To enter this literature requires formidable technical investment and many economists simply aren't equipped to follow even a fraction of it. There is then, of course, the temptation to criticize what one can't understand. But it does not excuse such economists from reading any of the many semi-technical and non-technical surveys that are regularly published in journals and books.

Economics has few possibilities of controlled experiment. Empirical work must mostly proceed by means of statistical inference on data given by history. Such inference not only requires theory but precise theory. It has been most successful in demand econometrics where the theory of choice caricatured by Eichner has been crucial. But it is a very difficult task and certainly we would not claim that the theory has watertight empirical support. To take another example ask how far high unemployment benefit is an important explanation of unemployment. One needs a pretty comprehensive theory of the economy and of individual choice before one can use such data as there are. The facts do not speak for themselves. Lastly it should be emphasized that a very large number of economists are engaged in empirical research. At the end of this we are left with an interesting question. It seems unlikely that *Nature* would have published an article in which, say, relativity theory had been attacked in way which made it obvious that the author had no glimmer of understanding of what the theory was about. Why is it that in subjects like economics any nonsense can get a hearing? Perhaps sociologists will have an explanation. □

Partha Dasgupta is Professor of Economics at the University of Cambridge and Fellow of St. John's College; Frank Hahn is Professor of Economics at the University of Cambridge and Fellow of Churchill College.

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Solar rotation as a function of depth and latitude

Timothy M. Brown

High Altitude Observatory, National Center for Atmospheric Research, PO Box 3000, Boulder, Colorado 80303, USA

A 5-day series of two-dimensional velocity images of the Sun is analysed to yield frequencies of solar p-mode oscillations with degrees between 8 and 50, with all azimuthal orders for each degree. The frequency splitting between modes with the same degree and radial order is related to the latitudinal variation of rotation, averaged over a depth range that depends on the degree. The observed splittings indicate that for $0.3R_{\odot} \leq r \leq 0.7R_{\odot}$, the solar latitudinal differential rotation is much smaller than at the surface (rotation roughly constant on spheres), and moreover that the rotation rate is close to the surface equatorial value.

How the solar rotation varies within the Sun has a bearing on many important questions in solar and stellar physics. For example, the solar rotation profile is crucial for testing theories of angular momentum transport within the Sun and other stars¹, current thinking relates the workings of the solar dynamo to the interaction between rotation and the solar convection², the depth variation of rotation may reflect the history of mixing episodes within the core³.

Measurements of solar oscillations⁴⁻⁶ have provided the first detailed information on rotation in the solar interior. Duvall and Harvey⁴ succeeded in measuring the frequency splitting between prograde and retrograde sectoral oscillation modes ($m = \pm l$, where l is the degree of the mode and m is its azimuthal order) for degrees between 1 and 100. These measurements give a clear idea of the depth variation of the solar rotation, but only at low latitudes, as these are the latitudes for which sectoral modes have large amplitudes. The observational method of Duvall and Harvey uses an optical integration along one dimension of the solar image; it is thus impossible to extend it to measure the frequencies of all m values for a given l . Full two-dimensional imaging of the Sun is necessary if one is to measure modes of all angular degrees and orders; without such information one cannot infer the latitudinal variation of the rotation.

The Fourier tachometer^{7,8} (henceforth FT) is a new instrument capable of making two-dimensional velocity images with the required precision and spatial resolution. I have used this device to undertake a series of observations that reveal at least some details of the latitude dependence of solar rotation.

Observations and analysis

Observations were taken for typically 9.5 clear hours per day on each of the days 8-12 June 1984. The observational cadence yielded one velocity image of the Sun per minute, with an image scale such that each pixel of the 100×100 Reticon detector spanned 19.5 arc s on the solar image. Typical velocity noise for a single pixel in the centre of the disk was about 6 m s^{-1} for a 60-s integration, or 15 cm s^{-1} for an integration over the entire solar disk. This is small enough to allow easy detection of the p-mode oscillations, which have typical velocity amplitudes (for a single mode) of 15 cm s^{-1} .

The data reduction first converted the raw velocity measurements to properly oriented maps of velocity residuals, with all unwanted Doppler shifts of known form subtracted out (some sources of instrumental bias, solar surface rotation and limb redshift, corrected for Earth's motion). Variations in the instrument's velocity zero point during a day were typically $2,000 \text{ m s}^{-1}$. To remove these variations it proved necessary to subtract a least-squares fit quadratic form in the image coordinates [effectively $\sin(\text{latitude})$ and $\sin(\text{meridian distance})$] from the data after all other reduction steps had been performed. This process destroyed the observations' sensitivity to oscillations with $l = 0-1$, and drastically reduced it for $l = 2-3$. These

reduction processes involved several interpolations to correct for guiding errors and for errors in the solar position angle. These undoubtedly degraded the spatial resolution and added to the noise. Moreover, due to uncertainties in the instrumental set-up, the correction for position angle (and hence the position of the solar poles within the reduced image) may have been in error by as much as 1° . After this stage of reduction, the residual velocity images had a typical r.m.s. velocity of 70 m s^{-1} .

The next step in the reduction was to apply spatial filters to the velocity residuals, producing the amplitudes in a spherical harmonic expansion for each time step. This was done for all l and m values for $0 \leq l \leq 50$. $l_{\text{max}} = 50$ was chosen primarily to limit computation time, as the spatial resolution of the data should allow measurements out to $l > 100$. The filtering method first interpolated the velocity measurements on to a grid of points equally spaced in solar longitude and in $\cos \theta$, where θ is the co-latitude. Fourier transforming in longitude then yielded amplitude as a function of m and $\cos \theta$, and finally Legendre transforms in the latitudinal direction gave the desired amplitudes as a function of m and l . Although this filtering method does not completely isolate the desired spherical harmonic from nearby ones with the same symmetry properties, it is probably only slightly worse than a truly optimal weighting technique. In the reduction of the June 1984 data, an error in the assignment of the radius of the solar image caused spatial filters that should have responded to spherical harmonics of degree l (the nominal degree) to respond instead to those of degree $1.03 l$. This also presumably increased the number of nearby spatial modes contaminating the response. In what follows, the degree of observed oscillation modes will mean the nominal degree, uncorrected for this scale error.

After the spherical harmonic decomposition, the time series for each l, m pair was Fourier-transformed and its power spectrum computed. These power spectra (about 2,500) were the basis for the subsequent analysis.

Observational results

The most useful representations of the power spectra are m - ν diagrams, which show power for modes of a given degree as a function of azimuthal order (m) and temporal frequency (ν). A fragment of one such diagram is shown in Fig. 1. The principal features visible in Fig. 1 are narrow, parallel concentrations of power ('ridges'), tilted with respect to the vertical axis. Each of these ridges corresponds to a multiplet of oscillations with particular values of radial order n and angular degree l , with azimuthal order m running from $-l$ at the top of Fig. 1 to $+l$ at the bottom. The ridges form broad clusters corresponding to different radial orders; in Fig. 1 the cluster below 2.9 mHz has $n = 13$, and that near 3.0 mHz has $n = 14$. The various ridges within each cluster result from the range of degrees to which the chosen spatial filter is sensitive, and from sidelobes resulting from diurnal gaps in the times series. Each true feature in the spectrum is flanked by a pair of sidelobes at a distance of

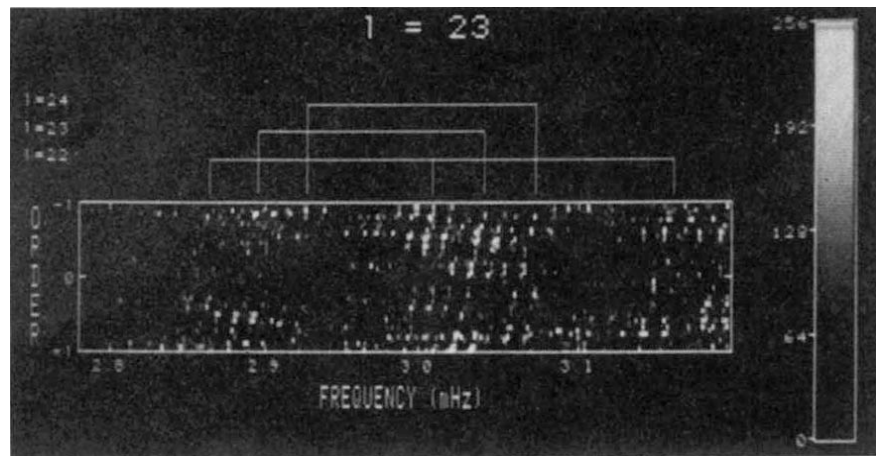


Fig. 1 Power (represented by intensity) as a function of temporal frequency for spherical harmonics with azimuthal order m , for a nominal degree l of 23. Degrees $l=22$ and $l=24$ are also present, as well as frequency sidelobes arising from gaps in the observed time series. Tick marks indicate the frequencies of oscillations with $m=-l$ for $l=22, 23, 24$. The maximum velocity amplitudes represented here are $\sim 10 \text{ cm s}^{-1}$.

$(1 \text{ day})^{-1} = 11.6 \mu\text{Hz}$, as well as weaker sidelobes at multiples of this distance. In Fig. 1, the marked ridges correspond to the central lobes for $l=22-24$. The intrinsic frequency resolution of the 5-day series is $2.6 \mu\text{Hz}$, but the apparent frequency width of the ridges is generally slightly larger than this. This additional width is mainly caused by interference from high-order sidelobes of other features in the spectrum; the effects of contamination from modes with neighbouring m values, additive noise, and finite mode lifetimes are probably less important for these data.

The variation of frequency within a multiplet is mainly a result of the solar rotation, although magnetic effects may also be partly responsible⁹. If the solar rotation frequency Ω (in cyclic units) were a function of radius alone, then the variation of frequency within a multiplet (as seen from the sidereal frame) would be given by

$$\nu(n, l, m) - \nu(n, l, 0) = -m\bar{\Omega} \quad (1)$$

where $\bar{\Omega}$ is an appropriate average of $\Omega(r)$ over the depth range sampled by oscillations with the given n and l . Thus, in the simplest approximation the effect of rotation is to make the observed frequencies linear functions of m . In the presence of latitudinal differential rotation, the dependence of ν on m becomes more complicated. If $\Omega(r, \theta)$ is small enough for centrifugal and Coriolis effects to be ignored, and if variations of Ω with $\cos \theta$ are negligible compared with those of P_l^m , then Cuyper's results¹⁰ may be used to write

$$\nu(n, l, m) - \nu(n, l, 0) = -m \frac{\int_{-1}^1 \bar{\Omega}(\theta) [P_l^m(\cos \theta)]^2 d \cos \theta}{\int_{-1}^1 [P_l^m(\cos \theta)]^2 d \cos \theta} \quad (2)$$

Because the integrals in equation (2) are symmetric in m , the frequency perturbations for slow rotation are always antisym-

metric in m . It also follows that the part of $\bar{\Omega}(\theta)$ that is antisymmetric about the equator has no effect on the measured frequencies. In what follows, I will consider only the symmetric part of $\bar{\Omega}(\theta)$. An axially symmetric magnetic field such as that discussed by Dicke¹¹ could produce frequency shifts that are symmetric in m , as could rotation that is rapid enough for centrifugal effects to become important. Thus, information of several sorts may reside in the variation of ν with m .

The principal difficulty in determining $\nu(m)$ for all degrees and radial orders is the very large number of oscillation modes that must be considered. Duvall and Harvey⁴ measured positions for individual peaks in their power spectra. With the hundred or so spectra they had to work with, this was a major undertaking; with 2,500 it is infeasible. To reduce the labour of estimating frequencies, I adopted a cross-correlation technique. For each m - ν diagram, I first constructed a spectrum averaged along a direction roughly parallel to the observed ridges. I then cross-correlated this average spectrum with the spectrum for each individual m value in turn, and adopted the position of the maximum of the cross-correlation as a measure of the frequency shift at that value of m . All cross-correlations were done over the ν range from 2.58 to 3.41 mHz; this frequency range spanned 4-6 different n values, depending on the value of l .

The cross-correlation method just described is easy to implement and has good noise-reduction properties, because all of the ridges in a given m - ν diagram (including temporal sidelobes and unwanted values of l) contribute to the result. This feature has some serious drawbacks, however. Averaging over different values of n and l entails a loss of depth resolution. Also, for degrees near 40, the frequency spacing between adjacent degrees becomes comparable to twice the sidelobe spacing. Thus, signals

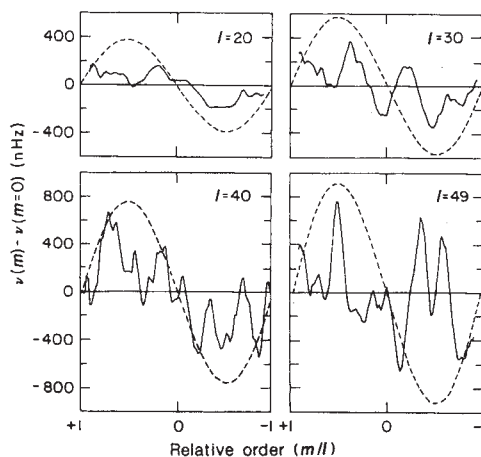


Fig. 2 Theoretical frequency shifts (dashed lines) that would result if the surface latitudinal differential rotation were independent of depth. They are compared with the observed shifts (solid lines) for several values of the nominal degree. A linear trend corresponding to a solid-body rotation with $\Omega = 461 \text{ nHz}$ has been subtracted from each curve. All observed curves are averages of three consecutive values of the nominal degree, centred on the indicated value, and all have been smoothed with a five-point running mean.

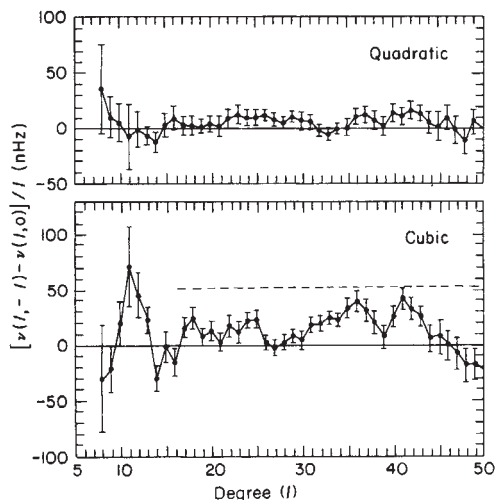


Fig. 3 Results of cubic fits to observed $\nu(m)$ relations, shown as a function of nominal degree. The raw quadratic and cubic fit coefficients have been multiplied by l and l^2 , respectively, so that the displayed values depend only weakly on degree. The dashed line in the lower panel is the cubic value that would result if the surface latitudinal differential rotation were independent of depth.

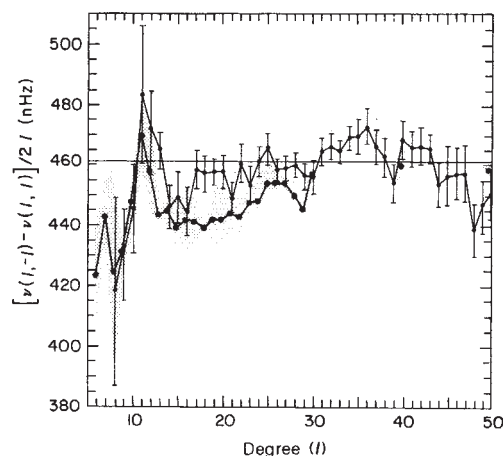


Fig. 4 Comparison between frequency splittings for sectoral modes derived from the present work (small circles with error bars) and from observations by Duvall and Harvey (large dots with errors indicated by stippling). See text for a detailed comparison.

from adjacent degrees overlap and interfere in unpredictable ways, adding significantly to the noise. Better data analysis techniques to be used in the future should eliminate the depth resolution problem, and minimize the importance of interference between adjacent degrees. As an interim measure, I suppressed obviously bad frequency measurements by applying a weighted polynomial fit to the data. A preliminary unweighted straight-line fit to the frequency shifts as a function of m provided an estimate of the standard error σ of the data. I then performed a cubic fit to the frequency shifts, with each point assigned a weight proportional to $\exp(-(\delta\nu/1.5\sigma)^2)$, where $\delta\nu$ is the difference between the measured frequency shift and the straight-line fit. In principle, it may be desirable to recompute σ based on the cubic fit and then iterate the fitting process until the results no longer change. In practice, σ was always substantially larger than the maximum contribution of the nonlinear terms in the fit, so iterating was unnecessary.

Figure 2 shows several examples of this fitting process. Each of the panels shows ν plotted against m/l averaged over three adjacent degrees, centred on the indicated degree. In each case, the frequencies have been converted from the geocentric to the sidereal frame by adding 31 m nHz to the measured values, and a straight line corresponding to solid-body rotation at $\Omega = 461$ nHz has been subtracted from the curves, emphasizing any differences between the observed rotation and that of a solid body. 461 nHz is approximately the observed rate of rotation of surface magnetic features at the equator¹². The dashed lines indicate the frequency variation one would expect if the latitudinal differential rotation observed at the surface were present at all depths. Note that spherical harmonics with $m = \pm l$ are concentrated near the solar equator, while those with $m = 0$ have largest amplitude near the poles. Thus, $[\nu(l, -l) - \nu(l, l)]/2l$ is largely a measure of the equatorial rotation rate, and $-d\nu/dm$ evaluated at $m = 0$ measures the rotation rate at high latitudes. The difference between these two values is a convenient parameter describing the latitudinal differential rotation, and I will sometimes refer to this as the differential rotation rate.

The most obvious feature of Fig. 2 is that the observed variation of ν with m is much more linear than one would expect from the surface rotation. To verify this impression, I have fitted cubics to the observed frequencies for all degrees between 8 and 50. Degrees < 8 are excluded because they have too few m values and too much noise to allow a useful fit. The dashed curves of Fig. 2 are well represented by cubics with no quadratic

term; large axially-symmetric magnetic fields may produce quadratic terms in the m - ν relation. Figure 3 shows both the quadratic and cubic terms in the fits plotted against degree, scaled to show $1/l$ times the contribution of each term to the frequency difference $\nu(l, l) - \nu(l, 0)$. Scaled in this way, the cubic coefficient corresponding to a particular value of the differential rotation is almost independent of l . The indicated error bars are twice the formal errors associated with the fits; the factor of two reflects an estimate of the systematic errors in the cross-correlation analysis, based on the difference between results of the cross-correlation analysis and an interactive fitting procedure, which I tested on a small number of ridges. The dashed line in the lower panel of Fig. 3 shows the magnitude of the cubic term implied by depth-independent latitudinal differential rotation at the surface rate. The errors in Fig. 3 are large for degrees < 10 because the fits are made to relatively few measured frequency points. For degrees ≥ 40 , the errors grow large because of the interference from adjacent degrees already mentioned. In view of the large formal errors (and probably larger systematic ones) for the higher degrees, the negative trend of the cubic coefficient above $l = 45$ may well be spurious. On the other hand, there seems no reason to doubt the small magnitude of the cubic term for $15 \leq l \leq 40$. Similarly, the large feature centred at $l = 11$ is probably real; a close examination of the ν measurements for degree 11 show nothing anomalous except the results of the fit.

The quadratic term plotted in Fig. 3 is everywhere consistent with a zero result, although for degrees larger than 20 the values are almost uniformly positive. This positive bias probably results from systematic errors in the analysis, though some solar process might be responsible.

A partial comparison between the observations reported here and the work of Duvall and Harvey⁴ may be effected by summing the linear and cubic terms in the $\nu(m)$ fits just described, and plotting the resulting values of $[\nu(l, -l) - \nu(l, l)]/2l$ as a function of degree. As the observations described here do not allow sufficient frequency precision when applied to single modes, this evaluation of the cubic fits represents the best available estimate of the equatorial rotational frequency splitting, simulating Duvall and Harvey's results. Both data sets are plotted in Fig. 4. The qualitative similarities between the two sets are striking. Both show a slow decrease in frequency splitting as l decreases from 40 to ~ 15 , and both show a large and localized increase in the splitting near $l = 11$, followed by a sharp decrease at smaller degrees. The Duvall and Harvey data also show an

increase in the splitting for $l < 6$. Unfortunately, the poor frequency resolution of the current data set prevents a useful comparison of the results for these low degrees.

Differences between the two data sets are also evident in Fig. 4. The clearest of these is that the splittings here are almost all larger than those from Duvall and Harvey, by an average of ~ 10 nHz. This is particularly true for degrees between 15 and 25, where a clear dip in the Duvall-Harvey values is scarcely discernible in the current results. The high noise and negative trend of the cubic coefficients for $l > 45$ are reflected in Fig. 4 as well as in Fig. 3. Although not in clear disagreement with the Duvall and Harvey data, results for these degrees should be considered suspect.

The splittings in Fig. 4 may also be compared with those measured by Hill⁶. The latter exceed those shown in Fig. 4 by more than a factor of 3; this disparity can hardly be reconciled except as a misidentification of oscillation modes in one or both cases. However, pictures such as those in Fig. 1 seem to leave little room for mode misidentification in the Fourier tachometer data set.

Conclusions

Barring unexpectedly large systematic errors, it seems clear from Fig. 3 that for $15 \leq l \leq 40$ the cubic term in the m - ν relation is smaller by a factor of 2-3 than one would expect if the surface differential rotation were present at all depths. Moreover, there is evidence that the cubic term decreases with decreasing degree. As l decreases, the oscillation modes penetrate deeper into the Sun; modes of degree 40 sample radii $\geq 0.6 R_{\odot}$, while those with degree 10 reach to $\sim 0.3 R_{\odot}$. Thus, the modes described here all penetrate at least to the bottom of the convection zone (assumed to lie at $0.7 R_{\odot}$). I believe that the most likely interpretation of the data is that an appropriately weighted average of the latitudinal differential rotation rate from the surface to $r = 0.6 R_{\odot}$ yields about half the surface value; averaging from the surface to $r = 0.4 R_{\odot}$ gives something less than one-third the surface value. An equally important point is that the decrease in differential rotation results mostly from an increase in Ω at high latitudes, rather than a decrease at low latitudes. Thus, the current data suggest that Ω at depth is more nearly constant with latitude than at the surface, and furthermore that Ω at all latitudes becomes similar to the surface equatorial rate.

The current data allow no strong statements about the variation of differential rotation through the convection zone, as all available modes are influenced by the Sun's outer layers in similar ways. However, the outer few per cent of the solar radius has a disproportionately large effect on the observed frequencies, because the low sound speed causes the pressure waves to spend a large fraction of their time near the surface. As the latitudinal differential rotation is large at the surface, the observed cubic term in the m - ν relation may result almost entirely from a thin surface layer, with the differential rotation small or absent through the bulk of the convection zone. I emphasize, however, that other interpretations are possible, and a definitive result must await better observations and a full analysis of the inverse problem.

The anomalous behaviour near degree 11 is a clear exception to the trends just discussed. For $l = 11$, the equatorial rotation

rate (as indicated by $[\nu(l, -l) - \nu(l, l)]/2l$) is larger than for nearby degrees, while the polar rotation rate (indicated by $-d\nu/dm$ at $m=0$) is smaller than for nearby degrees. The implied differential rotation is quite large, probably even larger than at the surface. Taken at face value, these facts suggest a shell of small radial extent near $r = 0.4 R_{\odot}$, with its equator rotating rapidly relative to neighbouring shells, and its poles rotating slowly relative to its neighbours. How such a localized rotational feature could be produced or maintained is far from clear. Indeed, it might arise from some unidentified observational error. An argument in favour of this possibility is that the internal error in the cubic fitting analysis for $l = 11$ is notably larger than for neighbouring degrees. Examination of the raw data shows that this error does not appear to be associated with a bad choice of fitting functions, but why the observational errors in this case should be particularly large is unknown. On the other hand, the appearance of large rotational splitting at $l = 11$ is common to both the current data set and that of Duvall and Harvey⁴. As the means used to obtain these two results were quite different from one another, an observational cause seems unlikely.

The quadratic term in the fit to $\nu(m)$ can be used to set an upper limit to the axisymmetric magnetic field in the solar interior. From Fig. 3, the maximum magnitude of the quadratic frequency perturbation for $l > 8$ is evidently about $10l$ nHz. Gough and Taylor⁹ computed the frequency changes caused by a particular magnetic field configuration similar to that suggested by Dicke¹¹. Using their results with geometrical factors appropriate to $l=9$ yields a maximum field strength of 1.3×10^7 G, depending on whether the field is assumed to go to zero at $0.7 R_{\odot}$ or at $0.3 R_{\odot}$. The quadratic frequency shift computed in this way is negative, in contrast to the bias in the quadratic coefficient shown in Fig. 3. This is perhaps another reason for believing that the apparent non-zero value for the quadratic term is spurious.

Finally, the discrepancy of typically 10 nHz between the equatorial rotational frequencies derived here and those reported by Duvall and Harvey requires explanation. Three possibilities, in (rapidly) decreasing order of probability, are: (1) there are different systematic errors in the two analyses. In particular, the incorrect radius value used in the current analysis should cause one to underestimate m by $\sim 3\%$. This leads to an overestimate of the frequency splitting by 3%, or ~ 13 nHz. Correcting this error should bring the two results into much closer agreement. Also, as mentioned above, the cross-correlation analysis used here yields results whose details should be considered suspect. (2) A cubic fit to $\nu(m)$ may not be adequate. A slow rotation zone, localized near the equator, could account for the observed disparity. Examination of curves like those in Fig. 2 shows no clear evidence for such an effect, however. (3) The solar rotation profile may have changed in the year between the two sets of observations.

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Cold dark matter, the structure of galactic haloes and the origin of the Hubble sequence

Carlos S. Frenk*, Simon D. M. White†, George Efstathiou‡ & Marc Davis§

* Astronomy Centre, University of Sussex, Brighton BN1 9QH, UK

† Steward Observatory, University of Arizona, Tucson, Arizona 85721, USA

‡ Institute of Astronomy, Madingley Road, Cambridge CB3 0HA, UK

§ Astronomy Department, University of California, Berkeley, California 94720, USA

A popular theory for galaxy formation holds that the Universe is dominated by exotic particles such as axions, photinos or gravitinos (collectively known as cold dark matter, CDM)¹⁻³. This hypothesis can reconcile the aesthetically pleasing idea of a flat universe with the standard theory of primordial nucleosynthesis and with upper limits on anisotropies in the cosmic microwave background⁴⁻⁶. The resulting model is consistent with the observed dynamics of galaxy clustering only if galaxy formation is biased towards high-density regions^{7,8}. We have shown that such a biased model successfully matches the distribution of galaxies on megaparsec (Mpc) scales⁹. If it is to be viable, it must also account for the structure of individual galaxies and their haloes. Here we describe a simulation of a flat CDM universe which can resolve structures of comparable scale to the luminous parts of galaxies. We find that such a universe produces objects with the abundance and characteristic properties inferred for galaxy haloes. Our results imply that merging plays an important part in galaxy formation and suggest a possible explanation for the Hubble sequence.

The morphology of most galaxies can be represented as a superposition of a rotationally supported disk and an ellipsoidal stellar system. The Hubble sequence classifies galaxies according to the relative importance of these components. Spiral bulges and faint ellipticals show substantial rotation, but most bright ellipticals rotate slowly¹⁰. Further, ellipticals are found preferentially in regions of high galaxy density whereas spirals avoid such regions¹¹. Spirals are surrounded by unseen massive haloes which are responsible for the flat rotation curves of their outer disks¹²; little is known about the mass distribution surrounding ellipticals. These systematic properties of galaxies and the associated characteristic scales are the least that any theory of galaxy formation should explain. Below we show that, at this level, a flat CDM universe provides an attractive arena for galaxy formation.

In a previous paper⁹ we studied gravitational clustering in model universes dominated by CDM. We found that a flat CDM model matches the observed galaxy distribution quite well if galaxies are assumed to form only at high peaks of the initial density field. Several biasing mechanisms have been proposed to inhibit galaxy formation elsewhere^{13,14}; although none of them seems particularly compelling, the formation of dark haloes should not depend on details of the biasing mechanism. To study halo formation, we have used the techniques and assumptions of our previous work to simulate the evolution of a cubic region of present size 14 Mpc from a redshift of 6 to the present day. The characteristic length scale associated with CDM fluctuations is proportional to $(\Omega H_0^2)^{-1}$ and their amplitude must be set by comparison with observations. Here and below we adopt $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$; this scaling and the initial fluctuation amplitude that we use are taken from our previous models, which had 10 times the linear scale. While various choices of parameters are possible, our adopted values not only lead to acceptable clustering properties but are also compatible with constraints on microwave background anisotropies and the age of the Universe. With this choice, there

are no further free adjustable parameters in the present simulation. The model contains $2 \times 10^{14} M_\odot$, which we represent by 32,768 particles. In the real Universe such a cube contains, on average, about 2 galaxies brighter than M31 and 10 galaxies brighter than M33. Our code models the interparticle force correctly on scales larger than 10^{-3} times the side of the cube. This resolution limit corresponds to 2 kpc initially and to 14 kpc at the end of the calculation.

The left-hand panels of Fig. 1 (labelled *a*) show the growth of structure in our model. By a redshift of 2.5, about 20 clumps with circular speeds exceeding 100 km s^{-1} have collapsed near high peaks of the initial linear density field. Of these, two have circular speeds $> 200 \text{ km s}^{-1}$. These clumps are aspherical and centrally concentrated. Between $z = 2.5$ and the present, some of them remain isolated and accrete extensive outer haloes, while others merge into larger systems. Figure 1 illustrates the evolution of three large clumps: one forms by a complex sequence of mergers, another is a binary in the process of merging at the end of the simulation, and the third evolves in relative isolation. The inner regions of the final systems are smooth and significantly triaxial; dynamical relaxation has effectively erased the structure of their progenitors. As a result of the large asymmetries present, these inner regions lose much of the angular momentum invested in the orbital motions of the subclumps to outlying material as substructure is destroyed. For example, the most bound 20% of the large cluster in Fig. 1*b* loses 70% of its angular momentum to the rest of the cluster between a redshift of 1 and the present day.

Figure 2 shows the circular velocity (V_c) as a function of radius (r) for the 10 largest systems in the simulation at the present day. These curves are remarkably flat at large radii and resemble the measured rotation curves in the outer parts of spiral galaxies. Observed rotation curves remain flat to considerably smaller radii than we can resolve with confidence in our calculation. However, unpublished work by J. E. Gunn, J. Barnes, G. R. Blumenthal and collaborators has shown that inclusion of the gravitational effects of the galaxy forming at the centre of the halo can result in extension of a flat rotation curve to the inner regions of the galaxy. Leaving aside the two largest objects in the simulation, two systems have amplitudes similar to that of M31 and the amplitudes of the others range down to that of M33. Prior to merging, the members of the massive binary had flat rotation curves with amplitudes of 240 and 260 km s^{-1} ; at the final time, their core regions have not yet coalesced. Their merged halo corresponds to the curve with an asymptotic velocity of 350 km s^{-1} in Fig. 2; much of the binding energy of the remnant was invested in the initial orbit of the pair. The slow rise within 60 kpc and the large amplitude of the top two rotation curves in Fig. 2 are uncharacteristic of even the largest spirals.

During the initial collapse of a clump, dissipative processes are likely to lead to the formation of stars in its densest regions on a dynamical timescale; this may result in a pressure-supported system similar to a spiral bulge, particularly if the clump has sufficient binding energy to prevent expulsion of gas by supernovae^{15,16}. Assuming 10% of the matter to be baryonic, dissipative collapse by a factor of ~ 5 is required to obtain a $2 \times 10^{10} M_\odot$ stellar system with a half-mass radius of 3 kpc. In agreement with previous suggestions^{17,18}, we argue that disk formation is likely to occur during extended periods of quiescent evolution, when high-angular-momentum gas from the outer halo can settle into centrifugal equilibrium. For the haloes in our simulation, contraction by about an order of magnitude is necessary to reach centrifugal balance. Disk formation may be terminated or prevented altogether by merging of the halo with other large clumps; the resulting objects may resemble S0 galaxies or faint ellipticals¹⁹. Bright ellipticals, on the other hand, are more luminous than the bulge of any spiral and must be formed in a different way. Formation by mergers can account both for their high luminosity and for their slow rotation. Thus, the large binary that merges near the end of the simulation can be interpreted as a pair of bright spirals in the process of turning

Fig. 1

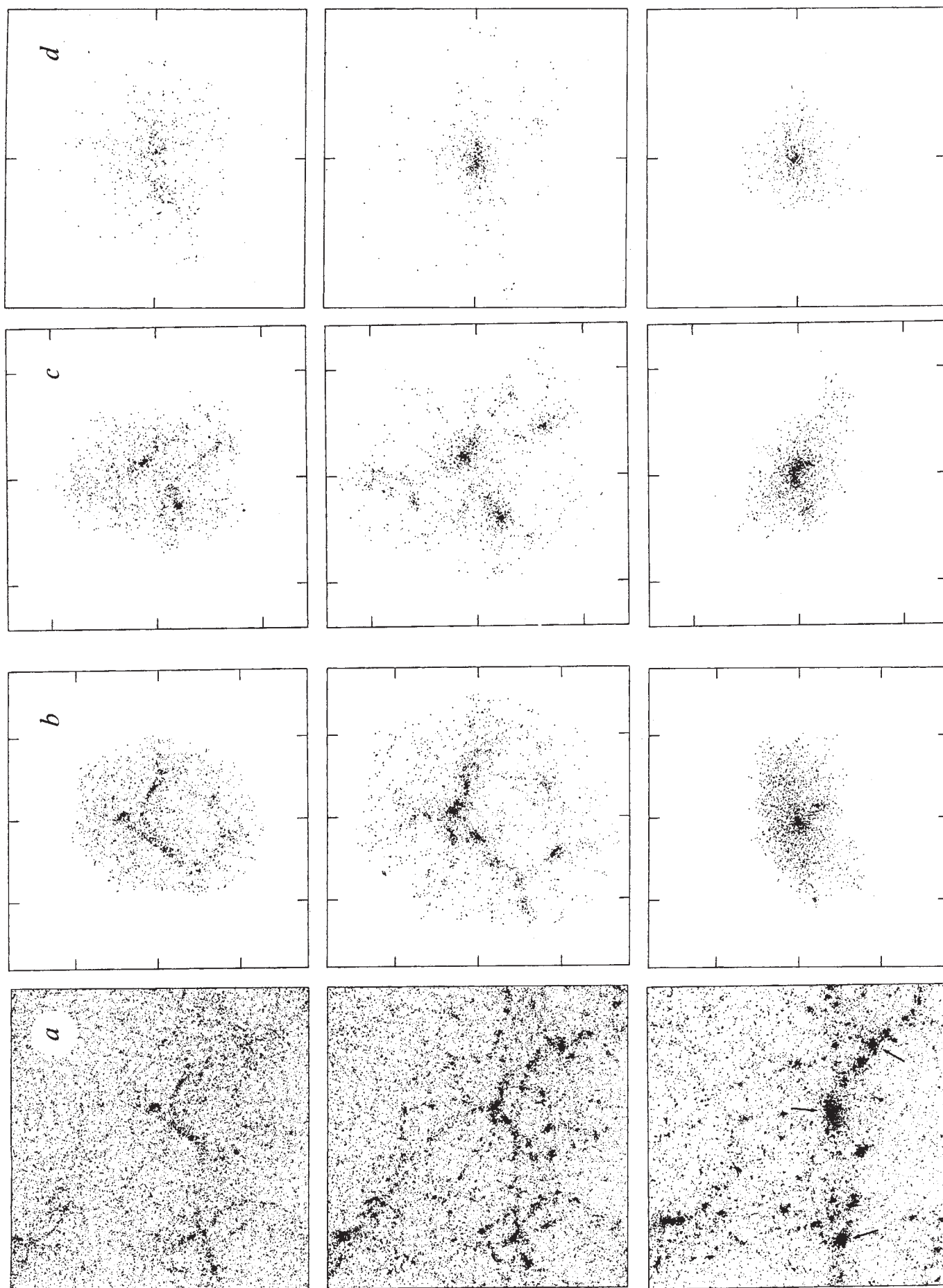


Fig. 1 (opposite) Evolution of a $(14 \text{ Mpc})^3$ volume of a flat CDM universe and of condensations which form in it. Time increases downwards in this figure. The upper, middle and lower sets of pictures are projections of particle distributions at redshifts of 2.5, 1.0 and 0, respectively. The column labelled *a* shows the simulation as a whole, with positions plotted in co-moving coordinates; the region shown at the top of this panel is thus 4 Mpc on a side. The group-finding algorithm described in our previous paper⁹ was used to isolate groups at a density contrast of ~ 500 at the final time. Three such groups are indicated by arrows at the bottom of *a* and the positions of their members at this and at earlier times are shown in *b-d*. They correspond to the two most massive groups in the simulation and to a more isolated system. Physical (not co-moving) coordinates are used for these plots; tick marks represent 1-Mpc intervals. Note the different type of substructure present at early times in each of these groups.

into an elliptical²⁰. In the largest object, substructure grows and then merges too rapidly for large disks to form. However, the stars in the cores of the individual subunits are likely to merge into a single ellipsoidal system. The central regions of the merged halo show little rotation. If these formation paths are typical of ellipticals, our models predict that such galaxies should be embedded in very massive haloes. However, the rotation curves of our two examples rise sufficiently slowly that their dynamical effects would not be apparent in stellar velocity dispersion measurements. Such haloes might be detected by X-ray observations of the hot gas they contain.

The mean density of our model is forced to be equal to the closure value; perturbations with a wavelength greater than 14 Mpc are neglected. Such fluctuations are responsible for the formation of clusters and voids in the real Universe and will affect galaxy formation. In a protocluster region, objects of high mass will form more rapidly than in our model, and in a protovoid they will form more slowly. The bias required to reconcile a flat universe with observations of the galaxy distribution must arise from a related modulation of galaxy formation. Merging and the tidal disruption of haloes occur most frequently in regions of high density. Thus, we envisage that bright and faint ellipticals will form predominantly in clusters, as is observed.

The major uncertainties in the galaxy formation picture that we propose stem from our ignorance of how stars form. In particular, we cannot demonstrate that dissipation and angular-momentum transport will lead to stellar systems with the binding energy and spin of observed galaxies. Another important issue which our present simulation cannot address is the dependence on mean density of the rate at which substructure is erased²¹. In our model, galaxy formation begins late in regions of typical density and continues until the present day. Observational searches for protogalaxies exclude a bright formation phase at

such recent redshifts²², but may not be incompatible with the protracted formation process that we envisage. Haloes with flat rotation curves may be produced in various cosmological scenarios, particularly if the behaviour of small-scale fluctuations resembles the CDM model. However, our simulation demonstrates that a flat CDM universe which accounts for the clustering properties of galaxies on megaparsec scales can, at the same time, form haloes with flat rotation curves and amplitudes similar to those observed. In addition, the structure and evolution of clustering in such a universe suggests attractive explanations for the origin of the morphological sequence of galaxies and for the correlation of morphology with clustering.

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A model for quasi-periodic oscillations in low-mass X-ray binaries

J.-M. Hameury*, A. R. King*† & J.-P. Lasota*‡

* Département d'Astrophysique Fondamentale, Observatoire de Paris-Meudon, F-92195 Meudon Principal Cédex, France

† Department of Astronomy, University of Leicester, Leicester LE1 7RH, UK

‡ Institut d'Astrophysique de Paris, 98 bis Bd Arago, F-75014 Paris, France

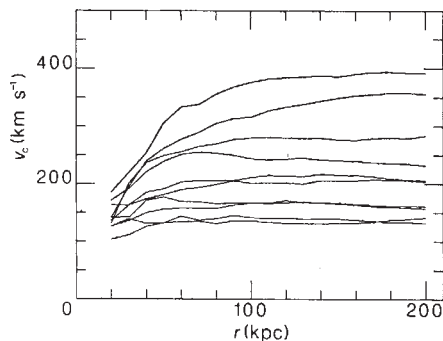


Fig. 2 'Rotation curves' for the 10 most massive clumps present at the end of the simulation. For each object circular velocities (V_c) were calculated as a function of radius (r) using $V_c^2 = GM(r)/r$ where $M(r)$ is the mass contained in a sphere of radius r centred on the densest region of the clump and G is the gravitational constant.

Quasi-periodic millisecond oscillations in the X-ray flux of two low-mass X-ray binaries, GX5-1 and ScoX-1, were recently reported¹⁻⁴. These quasi-periodic fluctuations are very similar to those observed in the soft X-ray flux of some dwarf novae in outburst^{5,6}. Guided by this analogy, we propose here that, as in the case of dwarf novae⁷, oscillations in low-mass X-ray binaries are caused by transient magnetic fields generated by turbulent dynamo action in the boundary layer and possibly in the corona of the accreting neutron star. The magnetic fields created in this way are too weak to channel the accreting material, but are strong enough to influence the heat transport in the neutron star corona and boundary layer and to cause bright spots with lifetimes of a few rotation periods to appear randomly on the neutron star surface. The rotation of this star's outer layers, together with the appearance and disappearance of the spots at random azimuthal positions, then gives rise to oscillations with the coherence characteristics reported in the observations.

Exosat observations of GX5-1 show intensity-dependent quasi-periodic oscillations between 25 and 50 ms, with a coherence time of typically 5-10 cycles. The amplitude of these pulsations is estimated to be $\sim 8\%$. In the case of Sco X-1, the period varies between 100 and 200 ms, with a reported amplitude of $\sim 3\%$. These values can be compared with those observed in soft X rays in the dwarf novae SS Cygni⁵ and U Gem⁶, which are 10 and 30 s, respectively, for the periods and $\sim 15\%$ for the amplitude, with a coherence time of a few cycles. The difference in the periods (two to three orders of magnitude) is obviously due to the fact that in dwarf novae, the accreting compact object is a white dwarf, while in low-mass X-ray binaries it is a neutron star, whose maximum allowed spin rate is much greater.

Another common feature of both classes of object is that coherent oscillations are not observed. This implies the absence of a permanent magnetic field strong enough to form a magnetosphere and channel the accreting material onto polar caps (that is, $\leq 10^4$ G for a white dwarf and $\leq 10^8$ G for a neutron star). The lack of observed coherent pulsations is a severe difficulty for models that seek to explain the quasi-periodic oscillations as a beat between the neutron star rotation frequency and the keplerian frequency at the magnetosphere^{1,8}.

It has been proposed⁷ that in the case of dwarf novae, the quasi-periodic fluctuations are due to transient hotspots at the surface of the differentially rotating external layers of the accreting white dwarf. In this picture, the period P represents the rate of rotation of the outer layers, and is determined by competition between accretion of angular momentum, and other torques (for example, viscous) linking the envelope to the core⁹. The hotspots are heated by conduction (along the magnetic field lines) of the energy dissipated in the boundary layer or a corona formed by thermal instabilities in this layer. The transient magnetic fields are generated by turbulent dynamo action in the differentially rotating outer layers of the accreting star. In this model, the magnetic field must have a minimum strength given by requiring that the Larmor radius of the electrons should equal their mean free path. This is smaller by many orders of magnitude than the field strength required to control accretion. There are obvious differences here from the earlier suggestions of Paczynski¹⁰. While in ref. 7 the magnetic fields are generated by surface dynamo action and thus transient, Paczynski assumes a permanent field linked to the white dwarf core, so that it is difficult to find a natural explanation for the frequent losses of phase coherence. Further, in that model, dynamical modulation of the boundary layer flow is postulated as the mechanism for producing bright spots on the stellar surface, rather than thermal conduction along field lines as in ref. 7. The model of ref. 7 can be naturally adapted to the case of a neutron star accreting from a disk; in the following, we shall use a different approach from ref. 7 in dealing with the dynamo problem. Our treatment is necessarily somewhat qualitative, as in particular a detailed investigation of the formation of coronae around non-magnetic neutron stars is required before quantitative estimates can be attempted. Our aim here is twofold: first to show that dynamo action is probable in the case of an accretion disk interacting with a non-magnetic neutron star, and second, to show that the main qualitative features of the observations do find natural explanations in our model. We outline briefly a possible observational test of the model.

In the boundary layer, the azimuthal speed varies from the keplerian velocity $\sim (GM/R_*)^{1/2}$ at the inner edge of the disk (where M , R_* are the mass and radius of accreting star and G is the gravitational constant) to the star's spin velocity on a scale smaller than the stellar radius. Because of the high rate of shear, one expects the boundary layer gas to be very turbulent. The joint presence of turbulence and differential rotation is very favourable for dynamo action. In particular, this situation is likely to produce a so-called $\alpha\omega$ dynamo^{11,12}, and we can use some of the results obtained by Pudritz^{13,14} in the slightly different context of thin accretion disks. Most of Pudritz's approximations simply require the azimuthal velocity to be much greater than the inward drift speed (very small Rossby number)

and are valid in our case. Taking into account the differences in the geometry, one can estimate the characteristic magnetic Reynolds numbers to be

$$R_\alpha = \frac{2}{5\pi} \frac{1}{A^2} \quad \text{and} \quad R_\omega = M_T^{-2} A \quad (1)$$

where it is assumed that turbulent diffusivity dominates magnetic diffusivity. A is a parameter smaller than unity equal to the characteristic azimuthal velocity in the turbulent layer divided by the keplerian velocity v_K ; M_T is the turbulent Mach number.

Dynamo action is possible if the dynamo number $R_\alpha R_\omega$ is greater than a critical value R_{crit} . The computation of R_{crit} requires a detailed knowledge of the structure of the turbulent layer; however, numerical and analytical computations usually give values between 5 and 100. Assuming here $R_{crit} \approx 10$, as in disks¹⁴, implies Mach numbers smaller than about $0.11 A^{-1/2}$. Observations and models suggest that M_T is in the range 0.1-1 (see, for example, ref. 15); this is compatible with the condition for dynamo action to occur.

According to Pudritz¹³, the maximum field strength is given by

$$\frac{B_{max}^2}{8\pi} \sim \frac{3}{8} M_T^2 P_{gas} \quad (2)$$

where P_{gas} is the gas pressure. This is of the order of the fluctuating kinetic energy density. Even if the actual field were three orders of magnitude lower than B_{max} , it would be strong enough to channel the heat flow conducted towards the star. The relevant timescale to establish the mean magnetic field in a dynamo action is $t_{dyn} \sim M_T^{-2} A^{-1} t_K$, where t_K is the Kepler time. Since $M_T \approx 0.11 A^{-1/2}$ for dynamo action to be possible, we have

$$t_{dyn} \geq 80 t_K \approx 8 \text{ ms} \quad (3)$$

for a $1 M_\odot$ neutron star, which is shorter than the observed periodicities as required for consistency of our model. It is not clear whether the mean field will be organized on very large scales (that is, $\sim R_*$) or whether it will, as a result of magnetic flux expulsion and magnetic buoyancy, form a few smaller structures (as in sunspots, for example). In both cases, a relatively strong field will be present in the corona, whatever the ratio between the gas pressure in the corona and the magnetic pressure, and it will conduct heat from the corona to one (or a few) hotspots at the surface of the neutron star.

In this picture, the main characteristics of the quasi-periodic oscillations of GX5-1 are explained in a natural way. The period is the rotation period of the spot(s) which emits the X-ray flux; the width of the peak in the power spectrum may be due to the fact that the spots are not always located at the same physical depth, and are therefore rotating at slightly different angular velocities. Finally, as in any model involving surface spots^{9,10}, the tendency of the period to shorten as the luminosity rises results from the fact that the accretion torques increase with an increasing accretion rate, and cause a stronger differential rotation of the outer layers of the neutron star. If one assumes, moreover, that the span of physical depths of the spots results from optical depth effects, then $\delta\Omega/\Omega$ is constant (where Ω is the local angular velocity) and so the width of the peak in the power spectrum increases with Ω and thus with increasing luminosity. The braking torque coupling the outer layers to the neutron star itself presumably varies with accretion rate, so that one can expect a more complex behaviour of the period in some cases.

Low-frequency (~ 1 Hz) noise is a common feature in the X-ray emission of all systems showing quasi-periodic oscillations, with, however, differing dependences on the source intensity¹⁻⁴. Surface spots at latitudes sufficiently high for them not to be eclipsed by the rotation of the neutron star would produce such noise, the lifetime of the spots giving the characteristic timescale. As the source intensity changes, the distribution of surface spots with latitude must change. There will also be a tendency for enhanced Compton cooling of coronal loops at high intensity to reduce the efficacy of conduction, thereby decreasing the brightness of the surface spots. The observable

effects on the quasi-periodic oscillations and low-frequency noise then probably differ between sources depending on the inclination of the neutron star rotation axis to our line-of-sight.

In the framework of our model, the neutron stars in both GX5-1 and Sco X-1 must be rotating at rates well below breakup (period ~ 0.1 ms). At typical accretion rates, $\sim 10^{-9} M_{\odot} \text{ yr}^{-1}$, this requires the systems to have lived no more than $\sim 10^8$ yr since the loss of any significant permanent magnetic field on the neutron star, which would previously have prevented spin-up.

A possible test of our model would be to try to measure the X-ray continuum spectrum of the quasi-periodic oscillations. This should be roughly black body and correspond to a radiating area somewhat smaller than the neutron star surface.

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A wide low-mass binary model for the origin of axially symmetric non-thermal radio sources

M. de Kool & E. P. J. van den Heuvel

Astronomical Institute, University of Amsterdam, Roetersstraat 15, 1018 WB Amsterdam, The Netherlands

The extended non-thermal radio sources G357.7-0.1 and G5.3-1.0 in the galactic bulge have about the same energy content in the form of relativistic particles and magnetic fields as the brightest ordinary supernova remnants (original energy input $\sim 10^{50.5}$ erg), but differ from the latter in having a marked axial symmetry^{1,2}. Their surface brightness gradually fades away towards one end of the symmetry axis, and at the opposite end one finds a compact radio source. The recently discovered radio source G18.95-1.1 (ref. 3) may belong to the same category. Helfand and Becker⁴ have argued that these structural properties can be explained most plausibly if these 'remnants' were produced by the ejection of matter with high kinetic energy by accreting binary systems at a rate $\gg 10^{37} \text{ erg s}^{-1}$, the ejection lasting for several times 10^5 yr while the systems were travelling through the interstellar medium with a speed of $\sim 100 \text{ km s}^{-1}$. We show here that the only type of neutron star binary that can fulfil both the condition of longevity and of a continuous high-mass transfer rate is a relatively wide binary in which the companion of the neutron star is a low-mass giant, with an orbital period of the order of weeks to months. Binaries of this type are expected to resemble closely the eight brightest galactic bulge X-ray sources^{5,6} as well as the progenitors of the two wide radio pulsar binaries⁷⁻⁹.

The arguments for an accreting binary model are⁴: (1) The only known types of sources that can inject large amounts of energy into non-thermal radio shells for an extended period of time are young pulsars and accreting neutron star binaries.

(2) The linear extents of the radio remnants measured along the axis are ~ 20 -30 pc. At a plausible speed of $\sim 100 \text{ km s}^{-1}$ this requires $> 2-3 \times 10^5$ yr travel time. (3) The same amount of time is required to obtain their $\sim 10^{50.5}$ erg energy content from a young Crab-like pulsar or a neutron star accreting at the Eddington limit (both having $dE/dt \approx 10^{38} \text{ erg s}^{-1}$). As the high energy-loss phase of a young pulsar ceases within $\sim 10^4$ yr after its birth, an accreting neutron star (or, possibly, a black hole) seems the only suitable candidate for providing the energy. (4) Those neutron-star binaries around which we infer the presence of relativistic particles (such as SS433 (ref. 10), Sco X-1 (refs 11, 12), Cyg X-3 (ref. 13) and Cir X-1 (ref. 14)) have rates of mass transfer near or above the Eddington limit ($\dot{m}_{\text{Edd}} = 1.5 \times 10^{-8} M_{\odot} \text{ yr}^{-1}$). (5) The presence of the compact radio source (of finite angular size) on the symmetry axis, which at least in the case of G5.3-1.0 is obviously connected to the radio remnant, suggests that continuous injection of energy and relativistic particles may still be taking place (see also ref. 15).

The possible absence of a strong X-ray point-source in or near the shells is not necessarily worrying, because in the case that the mass transfer rate exceeds the Eddington value, most or all of the X-ray emission may be blocked on its way towards us, by the excess matter which is piling up around the compact object, or in a thick accretion disk around this object. The energy generated by the accretion will then come out in other forms, presumably mostly as kinetic energy. Our example here is SS433, where the kinetic energy in the mass outflow at least equals the Eddington luminosity, while on the other hand the X-ray point source of SS433 has an X-ray luminosity of only $10^{35} \text{ erg s}^{-1}$, more than a factor of 10^3 below the Eddington value¹⁰.

In view of the arguments above, we adopt the accreting binary model and now examine which of the known types of accreting neutron-star binary systems can achieve a more or less continuous near or super-Eddington-limit mass transfer rate for several hundreds of thousands of years. The three main types of accreting neutron star binaries known in the Galaxy are: (1) the massive X-ray binaries, in which the companion to the neutron star has a mass $M_s > 8-10 M_{\odot}$; (2) the close low-mass X-ray binaries (orbital period ≤ 0.5 days), in which the companion to the neutron star is presumably an unevolved red dwarf; (3) the wider low-mass X-ray binaries (orbital period $P \geq 0.5$ days), in which the companion to the neutron star is an evolved low-mass giant ($< 1.2 M_{\odot}$) and the mass transfer is driven by the internal evolution of this star. Examples of the last category are⁵ Cyg X-2 ($P = 9.8$ days), 2S0921-63 ($P = 8.99$ days) and GX 1+4 (P at least several months, as the optical star is a low-mass M6 IIIe red giant).

The first two categories of systems are unable to maintain a high mass transfer rate for several times 10^5 yr, for the following reasons. The massive systems can reach a near- or super-Eddington rate only by means of Roche lobe overflow, which is, however, highly unstable as the mass transfer takes place from the much more massive to the less massive component, which causes the orbit to shrink rapidly^{16,17}. In these systems, the Eddington limit is reached in $\sim 5 \times 10^4$ yr on average (at most 10^5 yr) beyond which the transfer rate will increase rapidly (10^4 yr) to $\sim 10^{-4}$ - $10^{-3} M_{\odot} \text{ yr}^{-1}$ (see ref. 17) and a common envelope will form, presumably leading to rapid spiral-in of the neutron star and ejection of this envelope on a timescale $\sim 10^3$ - 10^4 yr (refs 16, 18-20). In the close low-mass systems, where gravitational radiation losses and/or magnetic braking are driving the mass transfer, it is very difficult to obtain mass transfer rates $\geq \dot{m}_{\text{Edd}}$ without coming into serious conflict with the observed luminosity distribution of X-ray sources^{21,22}. This makes these systems improbable candidates for having produced the radio shells.

On the other hand, the third category of systems, consisting of a neutron star and a less massive giant, seems ideally suited for fulfilling the required conditions: here almost any rate of mass transfer $\dot{m}_t$, below or above the Eddington limit, can be achieved for long periods of time (ranging from $\sim 10^5$ to 10^8 yr, depending on the value of $\dot{m}_t$). The transfer rate depends mainly

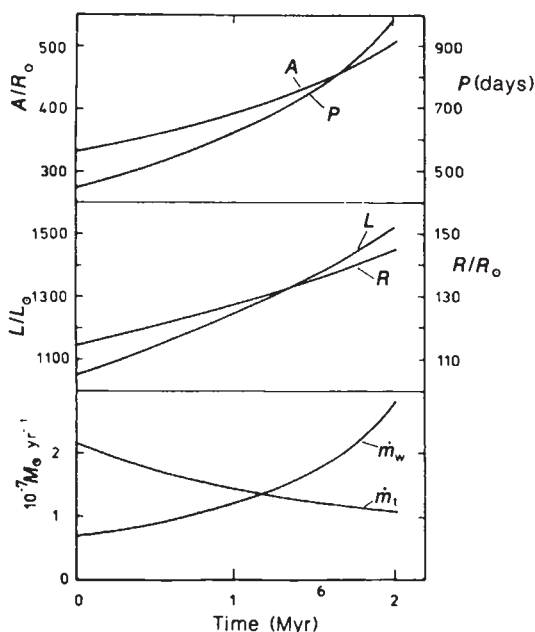


Fig. 1 Evolution of the mass transfer rate $\dot{m}_t$, wind mass loss rate $\dot{m}_w$ from the giant, luminosity L and radius R of the giant, orbital period P and separation A , for a binary system that started out with He-core mass $0.4 M_\odot$, $M_{\text{giant}} = M_\odot$ and a $1.4 M_\odot$ neutron star. The mass transfer lasts $\sim 2.0 \times 10^6$ yr and has an average rate $\sim 1.3 \times 10^{-7} M_\odot \text{ yr}^{-1}$ ($\sim 10 \dot{m}_{\text{Edd}}$), so that 90% of the transferred matter is ejected from the system.

on the initial orbital period and to a lesser extent on the initial mass and metal content of the companion⁵⁻⁷. Moreover, the mass transfer, even if it is super-Eddington, never becomes unstable as the transfer takes place from the less massive to the more massive component, which causes the orbit to widen. An additional merit of this type of binary model is that the location of the radio sources considered (near the galactic centre) is very similar to that of the bright galactic bulge X-ray sources, for which the model was originally devised and seems well established.

For conservative mass transfer (with conservation of total mass and total orbital angular momentum) from a giant with a degenerate He core, the mean mass transfer rate $\langle \dot{m}_t \rangle$ is in lowest order approximation given by (see ref. 5):

$$\langle \dot{m}_t \rangle = 5.3 \times 10^{-10} P [M_\odot \text{ yr}^{-1}] \quad (1)$$

where P is the initial orbital period in days. Equation (1) shows that to reach $\dot{m}_t \sim 3 \times 10^{-8} M_\odot \text{ yr}^{-1}$ ($= 2 \dot{m}_{\text{Edd}}$), the required initial orbital period is ~ 60 days. The duration of the mass transfer phase is roughly $M_{\text{env}}/\dot{m}_t$, where $M_{\text{env}} \approx 0.7 M_\odot$ is the envelope

Table 1 Mean mass transfer rate $\langle \dot{m}_t \rangle$, duration t_{RL} and final orbital period P_{fin} as a function of initial (degenerate) core mass M_c for wide binary systems initially consisting of a $1.4 M_\odot$ neutron star and a $1 M_\odot$ giant companion

M_c	P_{init}	With $\dot{m}_w^R$			With $\dot{m}_w^R/100$		
		$\langle \dot{m}_t \rangle$	t_{RL}	P_{fin}	$\langle \dot{m}_t \rangle$	t_{RL}	P_{fin}
0.30	65.01	2.4 (-8)	18.1 (6)	272	2.4 (-8)	23.1 (6)	405
0.40	450.6	1.3 (-7)	2.03 (6)	982	1.3 (-7)	3.63 (6)	1,335
0.45	852.0	2.3 (-7)	0.86 (6)	1,524	2.1 (-7)	1.84 (6)	1,897
0.60	2,114	2.5 (-7)	0.28 (6)	2,994	2.0 (-7)	1.30 (6)	4,282
0.70	3,921	4.0 (-7)	0.086 (6)	4,844	3.0 (-7)	0.66 (6)	6,055
0.80	5,822	5.5 (-7)	0.031 (6)	6,588	3.3 (-7)	0.34 (6)	7,432
0.90	7,670	7.0 (-7)	0.010 (6)	8,116	3.8 (-7)	0.14 (6)	8,531

P_{init} is uniquely determined by M_c . The degenerate cores with $M_c \leq 0.45 M_\odot$ consist of helium, those with $M_c \geq 0.60 M_\odot$ of C and O. The matter transferred in excess of the Eddington limit is assumed to leave the system with the specific orbital angular momentum of the neutron star. Mass transfer rates are in $M_\odot \text{ yr}^{-1}$, t_{RL} in yr and P in days. Numbers in parentheses indicate powers of 10.

mass of the giant, which for $\dot{m}_t = 2 \dot{m}_{\text{Edd}}$ yields $\sim 2 \times 10^7$ yr. Giants with a degenerate He core yield a maximum $\langle \dot{m}_t \rangle$ of $\sim 10 \dot{m}_{\text{Edd}}$, just before the He flash. A higher $\dot{m}_t$ ($10\text{--}100 \dot{m}_{\text{Edd}}$) can be reached by giants with a degenerate CO core.

The conservative assumptions used in ref. 5 are probably not applicable to the case of very high mass transfer rates considered here, as a considerable amount of mass (and angular momentum with it) is ejected from the system. We have therefore carried out calculations of orbital evolution and mass transfer rates similar to those in refs 5-7 for binaries initially consisting of a $1 M_\odot$ giant, either with a He core of mass $0.3 M_\odot < M_c < 0.45 M_\odot$, or with a CO core of mass $0.6 M_\odot < M_c < 0.9 M_\odot$, in which we did not assume total conservation as in ref. 5, but allowed for loss of mass and angular momentum from the system. We also included the effects of the mass loss due to the strong stellar wind expected from the luminous giants considered here. As we assume a jet-like ejection mechanism from the inner parts of an accretion disk, the mass which is transferred by Roche lobe overflow in excess of $\dot{m}_{\text{Edd}}$ leaves the system with the specific orbital angular momentum of the neutron star. The stellar wind carries the specific orbital and spin angular momentum of the giant. The method of calculation is based on the assumption that the orbit evolves in such a way that the giant's equivalent Roche radius remains equal to the radius dictated by its core mass. Together with our assumptions on the way mass and angular momentum are lost from the system, this uniquely determines the mass transfer rate as a function of time. The method is discussed in detail in ref. 5, so we shall not give detailed equations here but only list the M_c - R and M_c - L relations used, where R is the giant radius and L its luminosity. For the He-core giants these were (with $z = \ln(M_c/(0.25 M_\odot))$):

$$\ln(L/L_\odot) = 3.50 + 8.11z - 0.61z^2 - 2.13z^3 \quad (2)$$

$$\ln(R/R_\odot) = 2.53 + 5.10z - 0.05z^2 - 1.71z^3$$

and for the CO-core giants (with $y = \ln(M_c/M_\odot)$)

$$\ln(L/L_\odot) = 10.227 + 2.057y - 0.687y^2 + 2.282y^3 \quad (3)$$

$$\ln(R/R_\odot) = 6.771 + 1.290y - 0.332y^2 + 1.920y^3$$

Equations (2) are from ref. 5 assuming a Population I composition, since galactic bulge stars are known to have a rather high metal content, and equations (3) (S. A. Rappaport, M. de K. and E.J.P. van den H.) represent a fit to the curves calculated in refs 23 and 24. For the mass loss in the giant wind we used the expression by Kudritzki and Reimers²⁵:

$$\dot{m}_w^R = 5.5 \times 10^{-13} (R/R_\odot)(L/L_\odot)(M/M_\odot)^{-1} M_\odot \text{ yr}^{-1} \quad (4)$$

We also did calculations with $\dot{m}_w$ given by $\dot{m}_w^R/100$; an example of the results is given in Fig. 1, and all results are summarized in Table 1. Although the lifetimes of the systems considered here are shorter than those in ref. 5, these low-mass giant plus neutron star binaries are still able to provide the high (super-Eddington) mass transfer rates for periods $> 10^6$ yr, and thus can easily produce the radio sources.

We conclude that the wide binary model gives a good agreement with the present observations. Further work, especially a careful search for X-ray emission or detailed structure in the compact sources, could yield more evidence in this direction.

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Microbursts: a hazard for aircraft

P. F. Linden & J. E. Simpson

Department of Applied Mathematics and Theoretical Physics,
University of Cambridge, Silver Street, Cambridge CB3 9EW, UK

Microbursts are believed to have been responsible for several aircraft accidents in recent years. We report here laboratory experiments which show that when a descending column of dense fluid reaches the ground and begins to spread out horizontally, an intense vortex with a horizontal axis forms at the leading edge of the outflowing air. The intensity of this vortex results from the increase in vorticity due to the rapid stretching of the length of the leading edge. The properties of such a horizontal vortex with its associated updrafts and downdrafts are consistent with those found in microbursts which are a severe hazard to aircraft when taking-off or landing.

The crash of the TriStar on 2 August 1985 as it came in to land at Dallas-Ft Worth has been attributed to a small but intense thunderstorm outflow, called a microburst. Microbursts are produced by localized downdrafts of cold air which originate at high altitudes. The downdrafts are typically a few kilometres across, and can have vertical velocities $>10 \text{ m s}^{-1}$ (refs 1, 2). When the downcurrent reaches the ground the air is diverted horizontally, rather like the water from a tap hitting a horizontal plate, producing a radial outflow from the centre of the downdraft. Horizontal winds of 20 m s^{-1} are often produced by this spreading flow. In the Dallas disaster, gusts of up to 80 m.p.h. (35 m s^{-1}) were reported at the time of the crash. These intense flows have quite short lifetimes (2-5 min) and produce rapid changes of wind-shear in a small region.

A simple idealized picture of a downdraft^{1,2}, in the absence of significant synoptic winds, is shown in Fig. 1. The depth of the outflowing cold air is typically several hundred metres, so that an aircraft taking-off or coming in to land may fly directly into an area of strong wind-shear. Observing an increase in air speed the pilot will decrease the aircraft's forward thrust, and it is believed that the aircraft may stall when it enters the tailwinds associated with the radial flow on the far side of the downdraft.

With the high wind speeds involved, the parts of the leading edge of the cold air on either side of the downdraft will separate at, say, 40 m s^{-1} , and so during the short lifetime of a microburst these fronts will be 7-10 km apart. A microburst is thought of as a localized disturbance at the leading edge of the downdraft; in the case of the Dallas accident it appears that the aircraft hit the ground a few hundred metres after entering the microburst.

To explain some of the characteristics of observed microbursts, we introduce some laboratory experiments dealing with the spreading of dense fluid in a diverging radial gravity current. These observations, described below, suggest that the lift reduction experienced by the aircraft is caused by a strong wind-shear and downdraft produced by an intense horizontal vortex, which

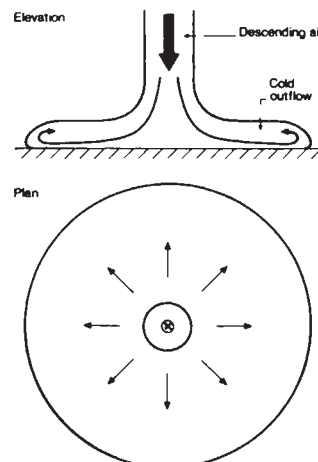


Fig. 1 A simple idealized picture of the descent of cold air from a thunderstorm. The hazard to aircraft is thought to be due to the variations of horizontal wind. The width of the descending air is $<4 \text{ km}$, and the depth of the outflow along the ground is a few hundred metres.

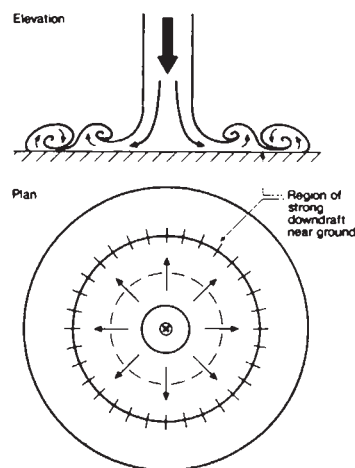


Fig. 2 The early stages in the development of a microburst. Most of the spreading mass of cold air is concentrated into an intense leading-edge vortex. This is sometimes followed by a second vortex. The scale is similar to that of Fig. 1.

itself is intimately linked with the front of the expanding ring of cold air. The horizontal scale of this vortex is comparable with the depth of the outflow and is much smaller than the overall horizontal extent of the cold air. A secondary vortex can follow the first and it is the variability in lift produced by the air currents in these vortices that produces the serious hazard to aircraft. The location of these vortices is shown in Fig. 2.

The outflowing cold air is an example of a diverging three-dimensional gravity current. The flow is driven primarily by the buoyancy forces produced by the density difference between the cold air and the warmer air surrounding it. In the case of a microburst, some of the initial horizontal momentum comes from the momentum of the descending air. Recently, successful numerical models of two-dimensional gravity currents at appropriate resolution have been run³. The essential feature of a microburst, however, is that the radially spreading outflow is three-dimensional. These flows have not yet been numerically modelled at a sufficiently fine scale in three dimensions and we turn to laboratory models for a description of the flow.

The flow is modelled in the laboratory using aqueous salt solutions with different densities to simulate the excess density of the cold air. The experiments produce, both qualitatively and

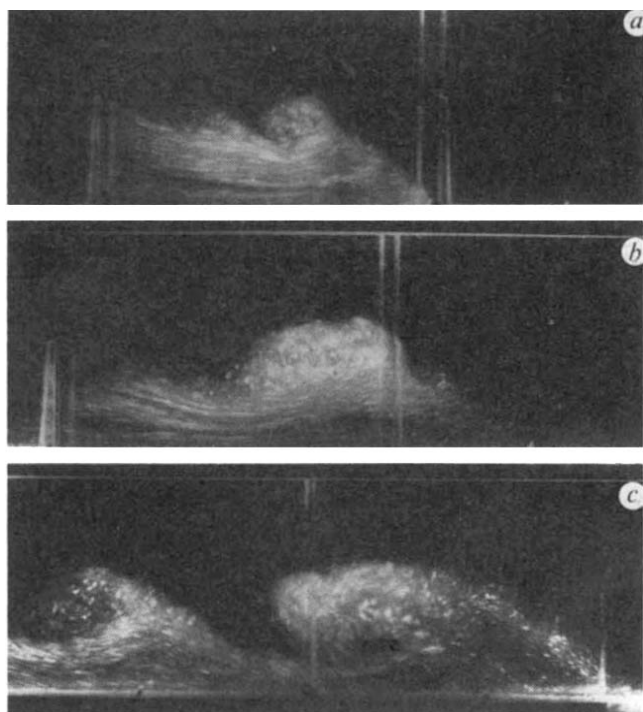


Fig. 3 Sequential streak photographs of the collapse and spread of a volume of salt water into a sector-shaped tank containing fresh water. The flow is from left to right. Note how the downdraft at the rear of the vortex at the leading edge penetrates almost to the ground in *c* as the dense fluid collapses. The vertical struts are at 30, 60 and 90 cm from the starting end of the tank, which is a sector of 10° angle. The photographs were taken at 2-s intervals and $g' = 12 \text{ cm s}^{-2}$.

quantitatively, the properties observed in two-dimensional atmospheric gravity currents^{4,5}. They show that the leading edge, or head, consists of a series of Kelvin-Helmholtz billows with associated vortex motions. In a two-dimensional current, these motions are restricted to the upper half of the current and do not penetrate to the ground.

An example of a rapidly spreading radial gravity current produced in the laboratory is shown in Fig. 3. In this case, there are two billows visible behind the leading edge, but in marked contrast to the two-dimensional current the billows temporarily occupy the full depth of the outflow. The vorticity in the billows has been increased by the radial spreading of the flow. This intensification results from the fact that the centre line of the vortex lies on a circle in plan view (Fig. 2), and so its length increases with time as the circle expands. Because the fluid volume in a vortex is (approximately) conserved, its cross-sectional area must decrease. Conservation of angular momentum about the centre line of the vortex then implies that the vorticity increases. The intensification is largest during the rapid expansion near the source and produces a leading-edge vortex which occupies almost the full depth of the dense fluid⁶.

A further demonstration of the intensification of the vortex by this stretching is shown in Fig. 4, where a steady gravity current in a channel of constant width encounters a section with diverging side walls. Once the current enters the diverging part of the tank the vortex is rapidly intensified and it becomes virtually cut off from the following flow. This experiment confirms that it is the lateral divergence of the flow that causes the descent at the rear of the leading-edge vortex to extend to within a short distance from the ground.

These experiments, although small-scale, have sufficiently high Reynolds numbers for viscous effects to be unimportant^{4,5}. The results agree with those obtained from larger-scale experi-

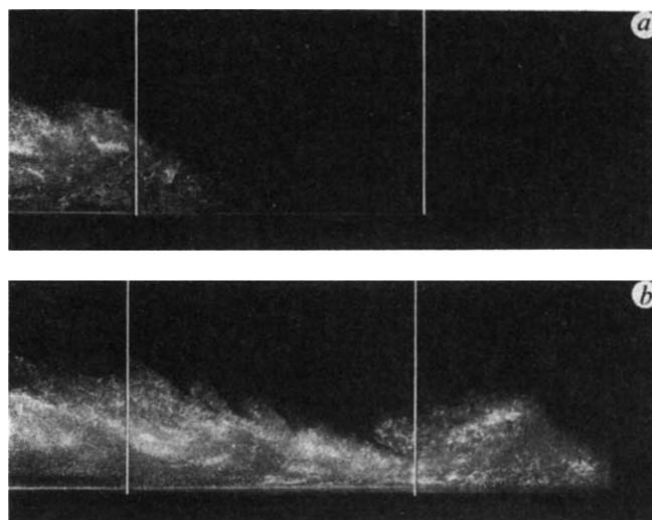


Fig. 4 The behaviour of the leading edge of a saline gravity current as it is allowed to increase its width. The width of the tank increases from 10 to 20 cm between the two white lines which are 30 cm apart. Upstream and downstream of this section the tank is of constant width. The front of a two-dimensional current is shown approaching the divergent part of the tank in *a*. Note how the leading-edge vortex has rolled up as the width of the front increases and it has reached almost down to the ground in *b*. The time interval between *a* and *b* is 5.7 s, and $g' = 14 \text{ cm s}^{-2}$.

mental releases of dense gas in the atmospheric boundary layer in which, in relatively calm conditions, an intense leading-edge vortex was also observed.⁷ The laboratory results suggest that the leading edge will propagate at a speed $U = 0.88(g'H)^{1/2}$, where $g' = g\Delta T/T$ is the reduced gravity associated with a temperature difference ΔT and H is the depth of the outflow. For a microburst with a temperature drop of 10 K and $H = 500 \text{ m}$, $U = 11 \text{ m s}^{-1}$. This velocity is less than the speed of the observed flows which suggests that the momentum of the descending air contributes to the strength of the outflow. In addition, the circulation of the descending air may further increase the intensity of the leading-edge vortex. All experiments to date have been conducted with dense fluid released from rest, and this is an area where further research is needed. But we emphasize that the enhancement of vorticity by lateral divergence will still be present, and is likely to be a strong effect.

The laboratory results (see Fig. 3) also show that the horizontal scale of the leading-edge vortex is comparable with the depth of the outflow ($\sim 1 \text{ km}$) and the velocities within it are of the order of the speed of advance of the leading edge ($\geq 20 \text{ m s}^{-1}$). A similar strong vortex has been described in measurements of the leading edge of a large-scale downburst by Doppler radar⁸. It is the wind-shear and downdraft at the rear of this vortex, occurring relatively close to the leading edge of the outflow, that are probably responsible for the danger of flying through a microburst.

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Opening mode of the Japan Sea inferred from the palaeomagnetism of the Japan Arc

Yo-Ichiro Otofuji*, Takaaki Matsuda†
& Susumu Nohda‡

* Department of Earth Sciences, Faculty of Science, Kobe University, Kobe 657, Japan

† Department of Geology, Himeji Institute of Technology, Himeji 671-22, Japan

‡ Kyoto Sangyo University, Kamigamo, Kita-ku, Kyoto 603, Japan

The western edge of the Pacific plate is marked by a chain of back arc basins stretching from the Aleutians, through Japan and the Philippines to New Zealand. Most of the basins lie behind volcanic island arcs far from continents and are the result of extension of the oceanic lithosphere¹. But the Japan Sea is bounded on one side by continental crust (the Sikhote Alin and Korean Peninsula of the Asian continent) and on the other by an active arc with continental fragments. It can thus be said to be associated more with continental rifting¹⁻⁴ than with oceanic processes. We report here palaeomagnetic data from the Japan arc which indicate that, since the early Miocene, north-east Japan has rotated counter-clockwise through 47° around a vertical axis while south-west Japan has rotated clockwise through 56° (refs 5-8). We attribute this differential rotation, which took place concurrently between 21 and 11 Myr ago, to the back arc opening of the Japan Sea⁸ with a 'double-door' mode, that is, there are two fan-shaped spreading basins facing each other. Such a fan-shaped opening is probably peculiar to the geological phenomenon of rifting of a continental fragment from thick continental lithosphere.

More than 200 oriented samples from 25 separate sites in 9 areas between 38.2° N and 40.3° N were collected from the Palaeogene to middle Miocene welded tuffs in north-east Japan. Tilting of the welded tuffs was identified by the orientation of eutaxitic structures. Their ages were determined by either K-Ar or fission track dating⁹, and the palaeomagnetic measurements were done at Kyoto University on standard 25-mm cores using a Schonstedt SSM-1A spinner magnetometer. All the samples were thermally demagnetized by stepwise heating in a zero field. The stable end points obtained during the demagnetization runs were used as the characteristic directions; statistical analysis for each site gave within-site circles of confidence with an average radius (α_{95}) of 4.0°. The tilting test was used to identify the primary component from the characteristic directions. The data from four sites which displayed secondary magnetization were excluded from further consideration.

The remanent magnetization, after thermal demagnetization and tilt correction of the welded tuffs with ages between 32 and

21 Myr, was characterized by a westerly declination, with a mean direction of $D = -41.2^\circ$, $I = 56.5^\circ$, $\alpha_{95} = 7.2^\circ$. The reliability of the westerly direction was ascertained through the presence of normal and reversed polarities. Tuffs aged between 14 and 11 Myr showed a northerly remanent magnetization ($D = -11.7^\circ$, $I = 60.5^\circ$, $\alpha_{95} = 15.3^\circ$).

Figure 1 shows the declination plotted as a function of age. The westerly declination of about 40° is maintained until 21 Myr, then changes to northward by 11 Myr. Because the declination values are obtained after tilt correction, the declination change during the Miocene shows the counter-clockwise tectonic rotation of the north-east Japan arc around the vertical axis.

The rotational motions of the north-east Japan arc and the south-west Japan arc^{6,8} are compared in Figs. 1 and 2. The timing of rotation of both arcs is concurrent: the difference between the climaxes of rotation of both arcs is <6 Myr. Differential rotation through more than 100° occurred between the north-east and south-west Japan arcs during the middle Miocene (between 21 and 11 Myr).

The palaeomagnetic inclinations before the rotation of the Japan arc (Fig. 2) suggests that north-east Japan, as is now the case, lay at higher latitudes than does south-west Japan. With the constraints that the declination of each arc before the rotation should be parallel to the palaeo-meridian¹⁰ and that the margins of the rifted arcs are fitted to continental margins before drift (the 3,000-m isobath being used to define the continental margin of the northern part of the Japan Sea), we conclude that the north-east Japan arc rotated counter-clockwise about a northern pivot (146° E, 44° N) through 50° and the south-west Japan arc rotated clockwise about a southern pivot (129° E, 34° N) through 54° (Fig. 3). The opening of the Japan Sea can thus be described as a 'double-door' mode in which two oceanic basins, the south-west and north-east sub-basins, were created concurrently. The opening mode of the south-west sub-basin is a typical fan shape because the spreading is associated with arc rotation around a pole position close to the arc. But the rotation pole for north-east Japan is quite a long way from the arc, resulting in a more parallel-sided fan shape for the north-east sub-basin.

This fan-shape mode of back arc spreading is rare. The major spreading centres in the back arc basins of the western Pacific are parallel to the bounding arcs, notably in Shikoku, Parece Vela and Lau basins and the Fiji Plateau. A spreading basin with a fan shape similar to the Japan Sea has only been found at the Balearic Basin in the Mediterranean¹¹. The Balearic Basin originated from the geological phenomenon associated with rifting and migration of the continental fragment constituting the Corso-Sardinian block from the Eurasian continent¹². The fan-shape mode is presumably characteristic of the rifting of a continental fragment from the mother continent.

The mode of opening seems to reflect the mechanical character of materials making up the lithosphere. The south-west and

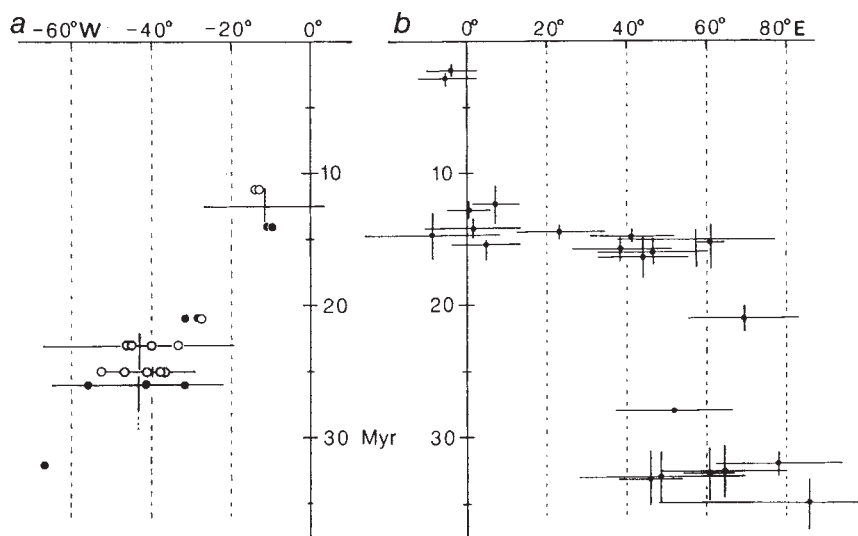


Fig. 1 Declination versus age for north-east Japan (a) and south-west Japan (b). a, Both mean declination data of sites and rock units are shown. The declination error bars of rock unit means are the α_{95} . ●, Data with normal polarity; ○, data with reversed polarity. b, Only the mean declination data of rock units are shown after ref. 8. The declination error bars are the α_{95} . Shaded declination zones are the declination with the α_{95} value of the mean direction of the period between 21 and 32 Myr for north-east Japan and between 30 and 35 Myr for south-west Japan.

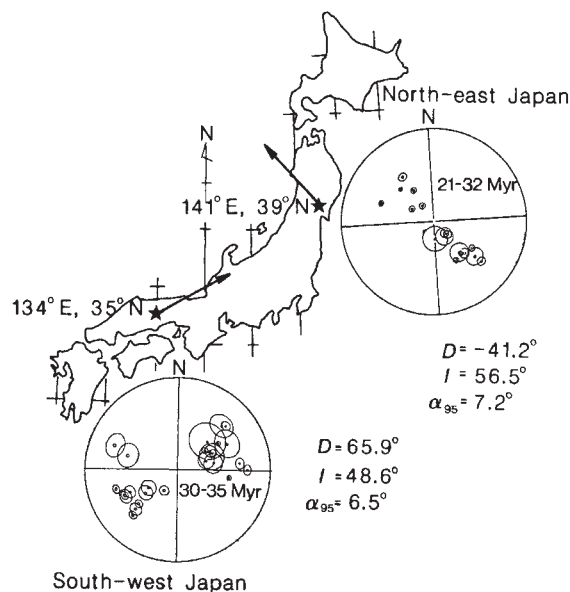


Fig. 2 Palaeomagnetic field directions and 95% confidence limits before rotational motion for north-east Japan and south-west Japan. Projections are equal area, solid symbols on the lower hemisphere, open symbols on the upper hemisphere. The data of north-east Japan have a period between 21 and 32 Myr, while those of south-west Japan have between 30 and 35 Myr. The mean directions are calculated as the value at the representative points (★) of north-east Japan (141° E, 39° N) and south-west Japan (134° E, 35° N), and the declinations of mean palaeomagnetic directions are indicated by arrows.

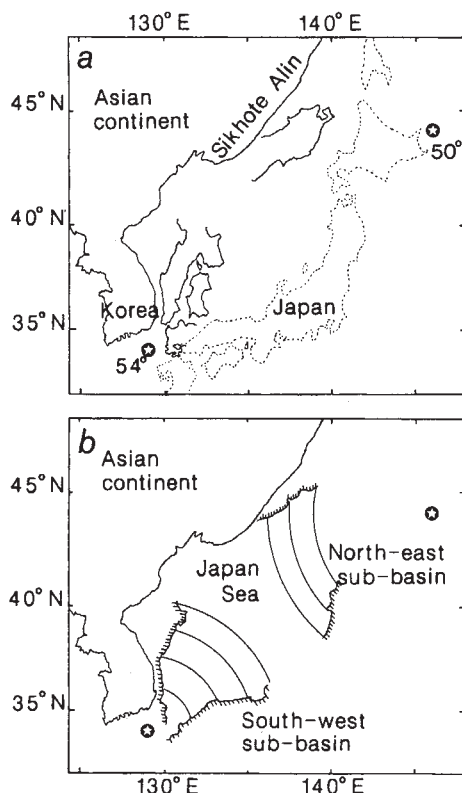


Fig. 3 Double-door opening mode of the Japan Sea. *a*, Palaeogeographic map of the north-east Japan and south-west Japan arcs before the rotation at 21 Myr. *b*, Possible opening mode for the Japan Sea. North-east Japan rotated about a northern pivot (146° E, 44° N) through 50° and south-west Japan rotated about a southern pivot (129° E, 34° N) through 54°. Both arcs rotated concurrently between 21 and 11 Myr. The clockwise rotation of south-west Japan is attributed to a typical fan-shape opening of the south-west sub-basin, while the counter-clockwise rotation of north-east Japan is due to a sub-parallel opening (a fan shape opening with a fairly parallel mode) of the north-east sub-basin.

north-east sub-basins in the Japan Sea are bounded on the continental side by the Korean Peninsula and Sikhote Alin, respectively. A thickness of 300 km is expected for the lithosphere under the Korean Peninsula, as the peninsula is part of the Sino-Korean Precambrian shield^{13,14}. Since Sikhote Alin is composed of Mesozoic and Cenozoic crust, the thickness of the lithosphere under Sikhote Alin is presumably thinner than that under the Korean Peninsula^{15,16}. On the other hand, a thickness of <100 km is estimated for the oceanic lithosphere of the western part around the Pacific margin from the Rayleigh wave study¹⁷⁻¹⁹. Thus the fan-shape mode forms as a result of the rifting of the thick and ductile lithosphere. As the lithosphere is thin and brittle, the spreading mode changes from a fan shape, through a sub-parallel to a parallel shape.

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Degassing-induced crystallization of basaltic magma and effects on lava rheology

Peter W. Lipman*, Norman G. Banks† & J. Michael Rhodes‡

* US Geological Survey, MS913 DFC, Denver, Colorado 80225, USA

† US Geological Survey, Hawaiian Volcano Observatory, Hawaii 96718, USA

‡ University of Massachusetts, Amherst, Massachusetts 01003, USA

During the north-east rift eruption of Mauna Loa volcano, Hawaii, on 25 March-14 April 1984 (Fig. 1), microphenocryst contents of erupted lava increased from 0.5 to 30% without concurrent change in either bulk magma composition or eruption temperature ($1,140 \pm 3^\circ \text{C}$). The crystallization of the microphenocrysts is interpreted here as being due to undercooling of the magma 20-30 °C below its liquidus; the undercooling probably resulted from separation and release of volatiles as the magma migrated 12 km from the primary summit reservoir to the eruption site on the north-east rift zone. Such crystallization of magma during an eruption has not been documented previously. The undercooling and crystallization increased the effective viscosity of the magma, leading to decreased eruption rates and stagnation of the lava flow.

The 1984 eruption of Mauna Loa provided an exceptional opportunity to observe the development of a large-volume basaltic a'a flow system for several weeks¹. Similar large a'a flows were last erupted on Mauna Loa in 1950 (ref. 2), before the availability of helicopter logistic support, devices for accurate temperature measurement, efficient radio communication,

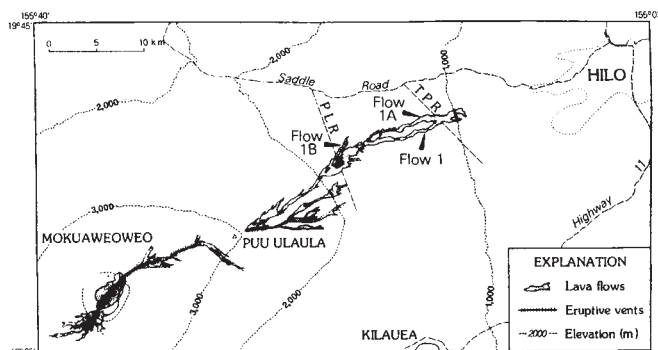


Fig. 1 Distribution of the 1984 flows, modified from ref. 9. All flows in the summit caldera (Mokuaweoweo) and adjacent upper north-east (NER) and south-west (SWR) rift zones were erupted during the first day of the eruption (25 March). PLR, Powerline road; TPR, tree-planting road.

abundant high-quality chemical data and other recent technological advances in monitoring techniques. In contrast, recent a'a flows from Kilauea Volcano^{3,4} have lasted only 1–2 days, were fed at rates an order of magnitude smaller than the 1984 Mauna Loa activity, and generally were inaccessible to observers except in vent areas and toes of flows (mid-zones are in heavily vegetated areas lacking helicopter landing points). Recent observations of flow dynamics at other volcanoes, notably Etna in Sicily and Arenal in Costa Rica^{5–8}, have also involved relatively small short-lived flows.

The 1984 Mauna Loa eruption commenced at the summit caldera (Mokuaweoweo: 4,160 m), extended into the upper south-west rift zone, and then migrated along the north-east rift zone to the 2,900-m level on the first day of the eruption. Activity during the remaining 21 days of the eruption was confined to lower vents on the north-east rift. The a'a flow system from the lower vents achieved a maximum length of 27 km within a few days of inception of the eruption (Fig. 1). Total eruptive volume⁹ was $\sim 220 \times 10^6 \text{ m}^3$. Eruption rates were as much as $2.9 \times 10^6 \text{ m}^3 \text{ h}^{-1}$ during the first 6 h of the eruption, stabilized at $0.4\text{--}1.1 \times 10^6 \text{ m}^3 \text{ h}^{-1}$ for the next 12 days, and slowly diminished thereafter (Fig. 2). As eruption rates declined, the main 1984 a'a flow (flow 1; Fig. 1) evolved from a simple narrow lobe with an efficient channel that delivered virtually the entire vent output to within 1 km of the flow toe, to an uprift-stagnating channel system characterized by levées, blockages, ponds and complexly branching overflows.

These changes in eruption rate and flow morphology occurred without significant variation in eruption temperature or lava composition, as documented by about 50 thermocouple determinations and 61 bulk-rock chemical analyses on vent spatter and channel lava. Temperatures of fluid lava remained within a narrow range of $\sim 1,140 \pm 3^\circ \text{C}$, and lava compositions were homogeneous virtually within analytical uncertainty (Table 1). The compositionally uniform 1984 flow is in marked contrast to lava erupted during Kilauea rift activity^{10,11} but is typical of Mauna Loa rift eruptions¹².

The only detected change in the 1984 lava is a more than 50-fold increase in crystallinity of quenched lava. Three distinct textural populations of crystals are present: (1) sparse resorbed phenocrysts or xenocrysts of magnesian olivine as much as 5 mm across, (2) dusty microlites $< 0.01 \text{ mm}$ long, and (3) microphenocrysts of augite, plagioclase and olivine commonly $0.05\text{--}1.0 \text{ mm}$ long. The sparse olivine phenocrysts, everywhere $< 0.5\%$ of the lava, are interpreted as disequilibrium crystals that were present in the summit magma reservoir before eruption. Microlites are absent in vent spatter and become increasingly abundant in down-channel samples; they record late groundmass crystallization of lava as it flowed down the channel. In contrast, the content of microphenocrysts in quenched vent samples increased

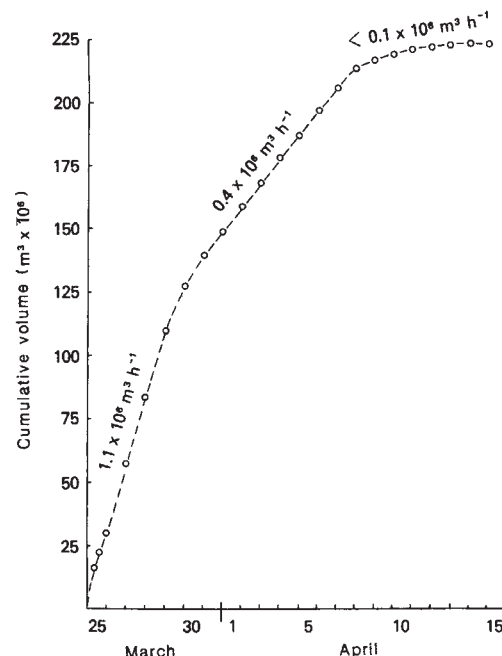


Fig. 2 Interpreted cumulative volume of 1984 Mauna Loa lava versus time¹.

during the eruption from $< 0.5\%$ of the lava during initial summit activity to as much as 30% during the waning eruptive phases (Figs 3, 4). Microphenocryst size increased concurrently with abundance, although measurement precision is lower¹. Microphenocryst size and abundance increased rapidly during the first days of the eruption, slowed during the subsequent two weeks, and increased again late in the eruption (Fig. 4). The pattern of changes in crystallinity is similar to that of eruption rates (Fig. 2); large changes occurred within a few hours after the beginning of the eruption on 25 March as the vents migrated from the summit to the 2,900-m level, but the abundance and size of microphenocrysts continued to increase after the main vent site stabilized. These changes occurred in the lava before eruption, as documented by quenched spatter samples, but the same microphenocryst populations are readily recognized in down-channel samples despite additional crystallization of microlites during surface flowage.

Most microphenocrysts in the 1984 Mauna Loa occur as skeletal, intergrown and radiating blades and plates (Fig. 3), including elongate, cored and swallow-tailed shapes that are typical of rapid crystallization from undercooled liquids^{13,14}. Such undercooled textures are especially prevalent in the relatively phenocryst-poor lava of early eruptive phases; late

Table 1 Chemical analyses (by X-ray fluorescence) of representative samples of the Mauna Loa 1984 lava (Fig. 3), and averages and standard deviations for all analysed samples

Date	25 March	25 March	29 March	14 April	Average, s.d.
Time	0145	0515	1750	0945	61 samples
Sample no.	MOK-1	NER-5	NER-12/23	NER-12/66	entire eruption
SiO ₂	51.51	51.56	51.71	51.27	51.54 ± .24
TiO ₂	2.08	2.09	2.08	2.10	2.08 ± .02
Al ₂ O ₃	13.75	13.69	13.69	13.60	13.65 ± .07
Fe ₂ O ₃ *	12.08	12.11	12.17	12.08	12.07 ± .06
MnO	0.17	0.18	0.17	0.19	0.18 ± .01
MgO	6.73	6.65	6.76	6.81	6.73 ± .08
CaO	10.57	10.55	10.57	10.43	10.50 ± .06
Na ₂ O	2.56	2.42	2.56	2.45	2.54 ± .11
K ₂ O	0.385	0.383	0.391	0.381	0.385 ± .009
P ₂ O ₅	0.24	0.25	0.26	0.24	0.24 ± .01
Total	100.13	99.87	100.38	99.55	99.92 ± .37

* Total iron listed as Fe₂O₃.

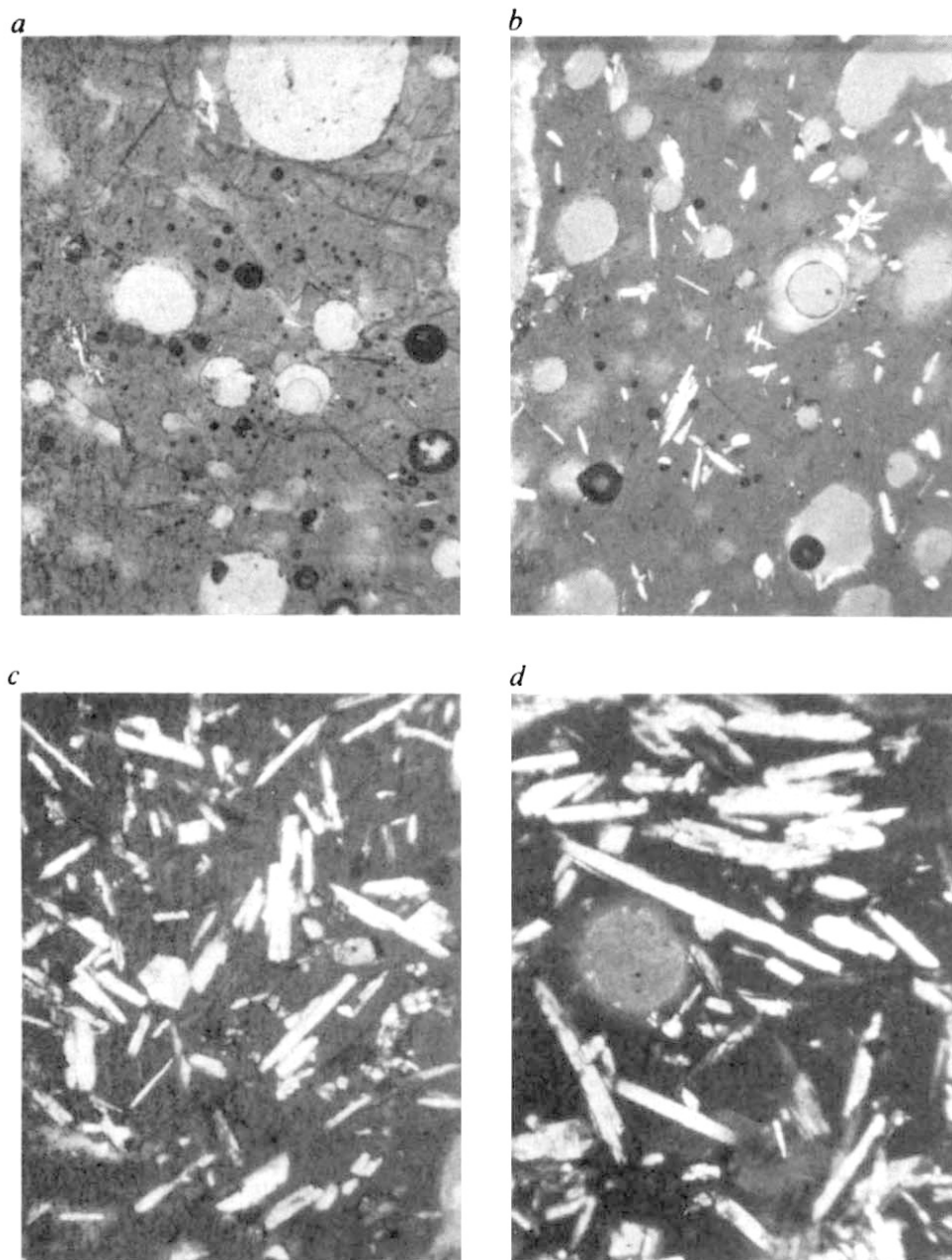


Fig. 3 Photomicrographs of representative 1984 Mauna Loa lava, showing progressive increase in size and abundance of microphenocrysts. Fields of view $\sim 1 \times 1.5$ mm. Microphenocrysts are clear rectangles (circular features are vesicular gas bubbles). *a*, 25 March, ~ 0145 h. Spatter from north-east side of 1949 cone, at 4,040 m. Sample MOK-1. 0.4% phenocrysts; 0.05 mm in average length. *b*, 25 March, ~ 0515 h. Spatter from upper north-east rift, at 3,650 m. Sample NER 5. 3.5% phenocrysts; 0.1 mm in average length. *c*, 29 March, 1750 h. Pahoe from perched pond (2,820 m). Sample NER 12/23. 21% phenocrysts; 0.3 mm in average length. *d*, 14 April, 0945 h. Channel overflow, near 2,870-m vent. Sample NER 12/66. 29.5% phenocrysts; 0.6 mm in average length.

eruptive phases have textures indicative of more prolonged equilibrium crystallization. We attribute the growth of microphenocrysts characterized by undercooled textures largely to separation of a gas phase during the eruption, not to a decrease in magma temperature. Observed eruptive temperatures remained constant throughout the eruption at $1,140 \pm 3^\circ\text{C}$, which is $20\text{--}30^\circ\text{C}$ below calculated liquidus temperatures for most phases at one atmosphere (olivine, $1,186^\circ\text{C}$; plagioclase, $1,169^\circ\text{C}$; and clinopyroxene, $1,146^\circ\text{C}$, all $\pm 15^\circ\text{C}$; using the method of Nielson and Dungan¹⁵). The observed magma temperatures would approximately coincide with the liquidus if the pre-eruptive magma contained 0.5–1.0% volatiles¹⁶. About 0.3–0.5% pre-eruptive volatile content has been estimated from gas data for tholeiitic basalts from Kilauea Volcano¹⁷, and $\sim 1\%$ volatiles for tholeiitic basalts in general¹⁸.

The virtual absence of microphenocrysts in much of the early-erupted lava on 25 March, followed by increases in the number and size of quenched microphenocrysts (Figs 3, 4), suggests that the magma chamber below the summit was initially sufficiently pressurized by volatiles to lower the liquidus to $\sim 1,140^\circ\text{C}$ and inhibit crystallization. With depressurization of the magmatic

system beginning at the onset of the eruption, separation of gas undercooled the magma without actually lowering the observed lava temperatures, thereby initiating precipitation of microphenocrysts. Degassing at the summit began at onset of the eruption and was later focused at the 3,300-m level, uprift of the active lava vents¹⁹. Degassing to atmospheric pressure was nearly complete by the time that magma reached the eruptive vents, even for early erupted lava, as indicated by volatile analyses of spatter.

Relatively minor undercooling can profoundly affect the nucleation and growth rates of crystals. At $20\text{--}30^\circ\text{C}$ of undercooling, crystals in basaltic melts can grow to sizes of 1 mm within periods as brief as a few hours^{20,21}. The only alternative to growth of microphenocrysts during the eruption—a zoned pre-eruptive magma chamber—seems less able to account for the compositional uniformity of erupted lava or the undercooled quench textures.

The volatile loss needed to initiate crystallization may have been small. Before eruption, basaltic magmas in the shallow reservoirs beneath the summits of Mauna Loa and Kilauea are believed to contain $<0.2\%$ CO_2 , 0.2–0.3% H_2O and 0.1% S (refs

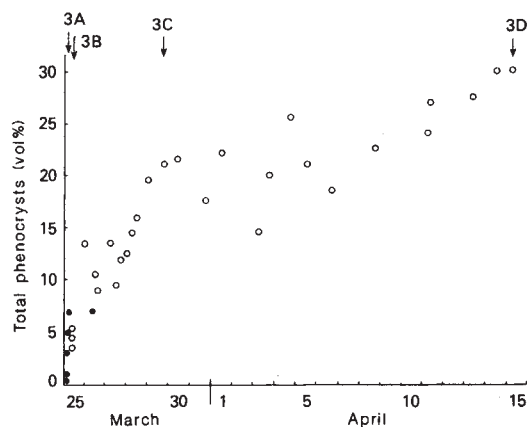


Fig. 4 Changes in abundance of microphenocrysts during the 1984 Mauna Loa eruption. Numbers at the top refer to samples illustrated in Fig. 3. Data are based on petrographic point-counting of thin sections, except for visual estimates of 25 March samples containing only sparse small microphenocrysts (<0.15 mm in average length). Values are believed to be accurate to within 10%; a major cause of uncertainty is the presence of microphenocrysts smaller in diameter than the thin section (0.03 mm). All samples are quenched glassy spatter and channel overflows. ○, Point count; ●, estimated.

17, 22, 23). Because of their relative solubilities, S and CO₂, but not H₂O, would have separated from the magma during transit from the summit reservoir, down the rift zone, to just below the vent. H₂O may have saturated and begun to separate only at depths as shallow as 100–200 m below the surface, if the rift magma was under lithostatic confining pressures. Although confining pressures within the upper kilometre or so of the fractured rift zones of Hawaiian volcanoes are unlikely to have been purely lithostatic, and at times approach atmospheric, these relations, nevertheless, cast doubt on the ability of H₂O loss alone to account for the undercooling and crystallization of the 1984 magma. High flow rates of the rift magma, efficient degassing and rapid crystallization would be required, even if the pressure on upper parts of the rift conduit were atmospheric. Alternatively, the major loss of S, beginning at the summit reservoir may have played a significant role in initiating crystallization. Unfortunately, little experimental data seem to be available to permit the evaluation of the role of small amounts of S on liquidus temperatures of basaltic magma.

Decreases in eruption rate, which characterize many basaltic eruptions, have been related to such factors as the release of elastic strain energy from magma stored in a subvolcanic reservoir, availability of magma from depth during the eruption, and conduit evolution from dyke to plug geometry²⁴. An additional factor that seems to have been important in the 1984 Mauna Loa eruption, and perhaps for others, is a change in magma rheology related to increasing crystal content of erupted lava.

The increase in microphenocryst size and abundance (Figs 3, 4) is roughly inverse to the decline in eruption rate (Fig. 2). The resulting increase in magma viscosity at the vent (10^2 – 10^3 Pa)^{1,25} was probably a major cause of termination of the eruption and the uprift stagnation of the channel. At ~1,140 °C, the apparent viscosity of tholeiitic basalt containing an equilibrium crystal assemblage (~25 vol. %) has been shown by field and laboratory measurements on Hawaiian basalt^{26,27} to be about one order of magnitude greater than an undercooled newtonian liquid lacking crystals. The changes in rheology, which are interpreted to have occurred largely in the pre-eruption magma chamber and rift conduits of Mauna Loa, are broadly similar to effects on the rheology of basaltic lavas inferred to result from degassing during eruption and downslope flowage²⁸.

Deviations from simple correlations between length of lava flows and eruption rates²⁹, demonstrated for Hawaiian flows³⁰,

may be due largely to effects of undercooling and increasing crystallinity of the lavas. Increased undercooling and crystallinity would promote higher effective bulk viscosity and yield strength in basaltic flows, causing transitions from pahoehoe to a'a and reducing the length of flows at any constant eruption rate. In contrast, eruption at liquidus temperature would promote transportation of pahoehoe in lava tubes, permitting longer-travelled lava flows. The rheology of lavas erupted as gas-rich foams (reticulite, in the case of Hawaiian basalts) that deflate during emplacement will be markedly different from that of degassed lavas; such contrasts may extend to highly silicic compositions³¹. Proposed simple correlations of viscosity and yield strength of lavas with chemical parameters such as silica content³² are similarly imperfect³³, perhaps due in large part to the complicating factors just listed.

Finally, note that the evolution of the 1984 flow system is similar in volume, duration and eruptive style to several previous historic (post-mid-nineteenth century) rift eruptions of Mauna Loa. Field and photogeological examination indicates that upslope stagnation of the distributary channel system characterized many of these earlier events, including the 1852 and 1942 flows that are adjacent to the 1984 flows. Sparse petrographic data indicate that other major historic rift flows (1843, 1926, 1935, 1942, 1950; north-east rift samples collected by J.P. and G. Lockwood) show trends of increasing microphenocryst contents during the eruption. Particularly significant would be similar observations for prolonged summit eruptions, such as 1940 or 1949, which could permit distinction between effects of depressurization of the summit reservoir, versus degassing along rift conduits.

More observations are needed on the interrelations between gas content, crystallinity, eruption temperature and viscosity of erupting basaltic lava. Does development of lava tubes and long-travelled pahoehoe require eruption temperatures near the one-atmosphere liquidus, or at least retardation of major degassing and attendant undercooling before eruption? If eruptions of undercooled lava, marked by increasing microphenocryst populations, can be shown to generate a'a flows close to the vent, observations of lava temperature and crystallinity can have important implications for assessing volcanic hazards during the early phases of major basaltic eruptions.

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Freshwater to marine-like environments from Holocene lakes in northern Sahara

J. Ch. Fontes*, F. Gasse†, Y. Callot‡, J.-C. Plaziat§, P. Carbonel||, P. A. Dupeuble¶ & I. Kaczmarzka†

* Laboratoire d'Hydrologie et de Géochimie Isotopique, Université Paris-Sud, F-91405 Orsay Cedex, France

† Laboratoire des diatomées, Ecole Normale Supérieure, F-92260 Fontenay-aux-Roses, France

‡ Institut des Sciences de la Terre, Université d'Oran, Es Sénia, Oran, Algérie

§ Laboratoire de Pétrologie sédimentaire et Paléontologie, Université Paris-Sud, F-91405 Orsay Cedex, France

|| Département des Sciences de la Terre, Université Bordeaux 1, F-33405 Talence, France

¶ Département de Géologie, Université de Rouen-Haute-Normandie, F-76130 Mont-Saint-Aignan, France

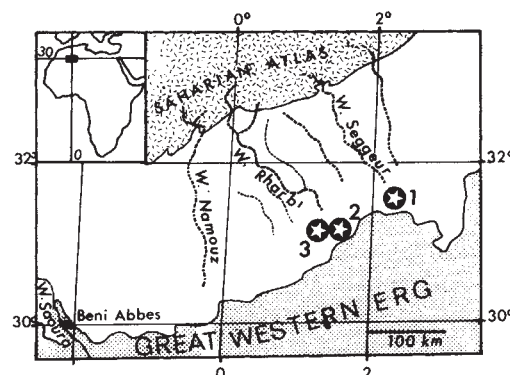


Fig. 1 Location of stratigraphic sections. 1, Hassi el Mejna; 2, Hassi Cheikh; 3, Daïet el Melah.

Continuous swamp and lacustrine sequences from the northern edge of the Great Western Erg are dated at 9,300–3,000 yr BP. Diatoms, ostracods, molluscs and foraminifera show great changes in salinity, ranging from freshwater to marine-like environments. The stable isotope composition of carbonates indicates that the evaporation of groundwater supplies is responsible for these fluctuations in salt contents. Until now, evidence for the occurrence of a wet climatic phase of early and middle holocene age in the north-north-west Sahara was based on a restricted number of ^{14}C dates from scattered lacustrine deposits and paleosols^{1–8}. The present study complements this knowledge and highlights the importance of local hydrological factors in interpreting palaeolimnological changes.

Stratified carbonaceous sediments lie in closed depressions of the hamada plateau. Some of them formed when dry valleys were blocked by the displacement of sand dunes in the Great Western Erg. At the present time, the water table of the aquifer is at about –50 m. The topography of the basins and the distribution of lacustrine sediments rule out the possibility of a surface outlet even during the high stands. Water losses were thus due to evaporation and/or seepage through the lake floors.

We summarize here the results obtained from dated stratigraphical sections (18 ^{14}C dates) taken from three individual depressions (31°–32° N, 1°–2°30' E: Hassi el Mejna, Daïet el Melah and Hassi Cheikh (Fig. 1). Supporting details, including percentage species analyses and isotope data, will be given elsewhere.

The accumulation of sediments is chiefly the result of chemical and biochemical precipitation of carbonates (calcite and aragonite; ~90%). The fraction $\leq 80\ \mu\text{m}$ is composed of idiomorphic and hypidiomorphic rhomboedra (~10 μm) of low-magnesian calcite (scanning electron microscope and X-ray diffraction analyses), which is considered to be caused by inorganic crystallization. Aeolian quartz grains are always present, with rare feldspars and very small amounts of clay minerals. Quartz and opal from diatom frustules represent about 10% of the bulk sediments which may also contain gypsum. The absence of bed load particles shows that water was supplied through the aquifer or wadi underflows as well as through local rainfall. However, a possible surface supply bearing detrital materials may well have been prevented by the filtering effect of a dense aquatic vegetation belt.

The excellent preservation of diatoms, ostracods and charophytes, the abundance of macrophyte remains, the presence of juvenile gastropods and the frequency of connected valves of the mollusc *Cerastoderma*, all denote very stagnant waters and preclude the reworking of the organisms.

The significance of radiocarbon ages obtained on lacustrine carbonates depends on the extent to which the total dissolved inorganic carbon (TDIC) reflects the ^{14}C content of the atmospheric CO_2 during precipitation. The radiometric age is con-

sistent when the TDIC is in equilibrium with the atmosphere, or when it is generated through the oxidation of recent organic matter. However, when a lake is supplied with TDIC by an aquifer or through a rise of deep-water CO_2 , the apparent age may be much older than the true age. Difficulties arise in interpreting the mixture of different sources of carbon or partial reequilibration with the atmosphere⁹. In our case, most of the dated samples could well include a quantity of old carbon since the lakes were supplied by groundwaters which may have been very old¹⁰. However, the radiocarbon ages of samples with ^{13}C content (+1 to +3‰) in or close to equilibrium with atmospheric CO_2 are consistent with those given by samples with much lower ^{13}C contents. Therefore, despite the fact that limestones occur in the Atlas watershed, we assume that all radiocarbon ages should be considered significant.

The inorganic calcite is depleted in ^{18}O (up to 4‰) with respect to coexisting molluscs (Fig. 2). This reflects an early stage of evaporation of discontinuous freshwater inputs rather than deviation from thermodynamic conditions induced by a 'vital effect'¹¹. Evaporation led first to calcite crystallization. A rapid concentration in ^{18}O then occurred while the molluscs secreted their shells. This indicates shallow waters and implies wide variations in salt content. The frequent juxtaposition, within a given level, of organisms which differ markedly in their salt requirements further attests to environmental instability. Both freshwater organisms and species usually found in marine coastal environments commonly occur in the same levels. The absence of saline regulation indicates that the rate at which salt was concentrated through evaporation was greater than the rate at which it was eliminated by seepage through the lake bottoms. However, the floor of the depressions comprises permeable sands. The poor drainage therefore reflects a water table close to the surface or intersecting it.

Besides the individual or seasonal episodes of freshwater input and subsequent evaporation, the studied profiles show different major tendencies and long-term fluctuations.

In the section of Hassi el Mejna (Fig. 2), ~4 m of lacustrine and swamp deposits lie on aeolian sands.

From 9,300 to <7,240 yr BP (samples 1012–1025), the carbonates display an apparent sedimentation rate of 0.8 mm yr⁻¹. At the base (samples 1012–1016), the diatom assemblages are rich in mesohalobous species (for example, *Cyclotella caspia*, *Cymbella pusilla*, *Nitzschia elegantula*). The presence of 'marine' forms (such as *Navicula zosteretii*) shows that the salinity was sporadically close to 35‰. The gastropod *Hydrobia*¹² and the monospecific ostracod population of *Cyprideis* gr. *torosa*¹³ reflect euryhaline conditions. The high ^{18}O contents (+1.27 to +3.58‰) show that the salinity is a result of the evaporation of solutions within the basin rather than the leaching of salts. These could easily be regarded as marine values. The ^{13}C contents are low, indicating that CO_2 was not a major factor in the calcocarbonic equilibria as it is in marine environments. Biological activity was the major source of dissolved carbon. Waters

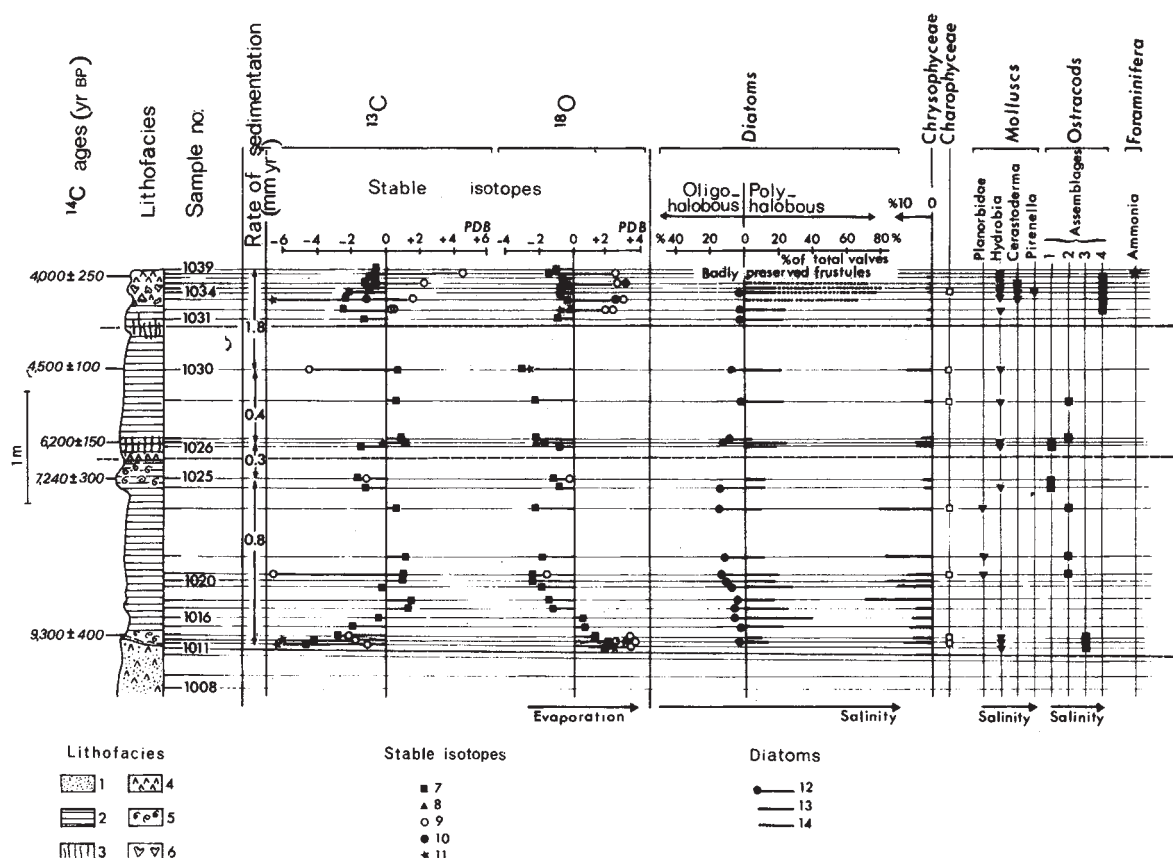


Fig. 2 Hassi el Mejna; changes in stable isotopes and fossil assemblages. Lithofacies: 1, sand; 2, stratified lacustrine carbonates; 3, traces of vegetation; 4, gypsum; 5, gastropod shells; 6, *Cerastoderma* (bivalve). Stable isotope content, measured on: 7, mineral fraction $\leq 80 \mu\text{m}$; 8, charophytes; 9, gastropods; 10, *Cerastoderma*. Diatoms: 12, oligohalobous species known from fresh water only (*Cymbella laevis*, *C. kappii*, *C. microcephala*, *C. cesatii*, *Gomphonema* spp., *Synedra ulna*); 13, taxa usually found in coastal marine environments (*Amphora arcus* v. *sulcata*, *Actinocyclus subtilis*, *Mastogloia aquilegia*, *Mastogloia baltica*, *Mastogloia braunii*, *Navicula pseudocrassirostris*, *Navicula zosteretii*, *Entomoneis decussata*, *Cyclotella stylorum*, *Synedra tabulata* + v. *Synedra pulchella*); 14, *Amphora tenerrima*, a diatom from marine and meso- to hyperhaline environments; euryhalobous, mesohalobous and halophilous are excluded from the diagram. Chrysophyte content (given as the ratio of the number of Chrysophyte cysts to diatom valves), an additional index of freshwater environments. Ostracod assemblages: assemblage 1: *Cyprideis* gr. *torosa*, *Candona* gr. *neglecta*, *Cypridopsis* spp., *Darvinula* spp.; assemblage 2, as for assemblage 1 + *Limnocythere* sp.; assemblage 3, *Cyprideis* gr. *torosa*; assemblage 4, *Cyprideis* gr. *torosa*, *Loxoconcha elliptica*.

remained stratified. From samples 1016 to 1023, a clear dilution is revealed by the increasing percentage of oligohalobous diatoms (for example, *Cymbella laevis*, *Cymbella kappii*). The abundance of epiphytic diatoms suggests the development of a dense aquatic vegetation. Freshwater gastropods¹² (Planorbidae with thin shells) replace *Hydrobia*. The ostracod fauna consists of the eurytopic *Cyprideis* gr. *torosa* (dominant) which is associated with the freshwater species *Candona* gr. *neglecta*, *Cypridopsis* sp. 1, sp. 2, *Darvinula* sp. 1, sp. 2, and with the alkaliphilous *Limnocythere* sp. in samples 1021–1024. This dilution is also supported by lower ^{18}O contents (–2.34, –1.47%). The ^{13}C contents increase because of a stronger influence of atmospheric CO_2 . This may be the result of several factors: the lower production of biological CO_2 , higher pH, better mixing, higher residence time or shallower water. In samples 1024 and 1025, freshwater diatoms and molluscs tend to disappear although ostracod assemblages and ^{18}O contents still reflect rather dilute conditions. After sample 1025, a desiccation is suggested by the intercalation of a thin gypsum layer, the disappearance of several diatom species (for example, *Synedra filiformis*, *Cyclotella stylorum*) and by the drop in the apparent sedimentation rate between samples 1025 and 1027 (0.3 mm yr^{-1}).

From >6,200 to 4,500 yr BP (samples 1026–1029), an environmental evolution very similar to that observed in samples 1012–1025 is shown by the successive diatom assemblages, fauna communities and stable isotope compositions. The maximal dilution appears in samples 1027 and 1028.

Between <4,500 and 4,000 BP (samples 1030–1039), a drastic

change occurs in the hydrological balance, and leads to an increase in salinity. 'Marine' diatoms (such as *Entomoneis decussata* and *Actinocyclus subtilis*) and *Amphora tenerrima*, which tolerates hyperhaline environments, become predominant. The ostracod assemblage with *Cyprideis* gr. *torosa* and with the thalassic form *Loxoconcha elliptica* reflects mesohaline to hyperhaline conditions. The mollusc *Cerastoderma glaucum* appears in sample 1035 and is followed by *Pirenella conica*, never previously recorded at such a distance from the marine coastline. Foraminifera (*Ammonia tepida*) are present in sample 1039. At this point, ^{18}O and ^{13}C contents reach their highest values (+3% and 4.48%, respectively). The unexpectedly high ^{13}C value recorded in *Cerastoderma glaucum* (+4.48%; +2.7% after appropriate normalization¹⁴) reflects that of the total dissolved inorganic carbon, given that *Cerastoderma* secretes its calcite at or close to equilibrium with the TDIC¹⁵. This may be a result of methane formation in stratified waters with a reduced environment in the bottom sediment, or of cool conditions.

Carbonate deposits (~1.5 m thick) found in the section of Daïet el Melah include numerous shelly levels, dated between 7,400 and 5,400 yr BP. Towards the centre, the depression is occupied by concentric layers of evaporites (gypsum and halite) which mark the regression stage(s) and confirm that vertical permeabilities are low. At their base, the sediments contain freshwater charophytes, euryhalobous gastropods (*Hydrobia* and *Melanoides tuberculata*) and a mesohalobous ostracod community (*Cyprideis* gr. *torosa*, *Loxoconcha elliptica*) which is constant throughout. Evaporation was moderate (^{18}O contents

+0.55, +0.88). Increasing salinity is then documented by the appearance of the thalassic molluscs *Cerastoderma glaucum* and *Pirenella*. The top layer contains two highly euryhalobous species of foraminifera *Ammonia tepida* (about 95%) and *Trichohyalus* sp. aff. *aquayoi*. Oxygen-18 contents are high (+3.06, +3.16). The salinity was close to that of sea water. Carbon-13 values indicate that the environment was influenced by biogenic CO₂ at the base and tended to equilibrate with the atmospheric reservoir of CO₂ towards the top.

In sections of Hassi Cheikh, shell-rich sediments are available from several small outliers (~40 cm thick). Four are dated at 3,280–3,080 yr BP. Despite this narrow time span, large fluctuations of environmental conditions can be observed. In one outcrop, brackish water diatoms (*Campylodiscus clypeus*, *Achnanthes grimmei*) and even 'marine' species (*Navicula pseudocrassirostris*) coexist with oligohalobous taxa (*Cymbella microcephala*, *Achnanthes minutissima*, *Cocconeis placentula*) and become dominant towards the top. Molluscs (*Bulinus*) are limited to juvenile forms, suggesting an environmental instability which may have inhibited their full development. The presence of a monospecific population of the mesohalobous ostracod *Cyprinotus* sp. seems to imply that salinity is responsible for such an inhibition. The ¹⁸O content of the inorganic calcite remains low (−3.69 to −3.01). These features may be accounted for in two ways: either sporadic supplies of very fresh waters which evolved rapidly through the evaporation of shallow water, or supplies which dissolved a preexisting crust of salts left behind after the previous flood had evaporated.

Very low salinities prevailed during the deposition of two other synchronous outcrops. Diatom assemblages (with freshwater epiphytes dominant), mollusc fauna (*Bulinus truncatus*, *Gyraulus* sp. aff. *ehrenbergii* and *Biomphalaria* sp. aff. *sudanica*) and charophytes are indications of oligohaline swamps with dense vegetation. As expected, the ¹⁸O content of inorganic carbonate is low (−4.96 to −1.21). The ¹³C content (−1.18 to +1.87) indicates a predominance of the atmospheric control of TDIC, that is, well-mixed waters and low primary production.

Deposits from Hassi Cheikh exhibit the lowest recorded salinities. However, no aquatic environments were previously reported at this latitude around 3,000–3,200 yr BP, which was regarded as an arid period^{15–18}.

The study area is located more than 500 km from the coast at an elevation of about 500 m. Obviously, any marine intrusion in this region during the past 10,000 yr is excluded. However, the deposits have fossils and stable isotope contents which, had they been found elsewhere, might have been attributed to marine environments or to coastal lagoons connected with the sea. We have shown that it is possible for an initial freshwater supply to evolve and mimic marine facies when the evaporation makes the salinity increase up to marine values. The biotopes are readily colonized by euryhaline and possibly stenohaline 'marine' organisms after being transported stage by stage through environments with differing physicochemical properties, thus leading to ecological selection. Caution is therefore essential when invoking connections with the marine domain from the occurrence of such 'thalassic' or 'thalassoïd' ecosystems.

Agreement between the fossil (especially diatom) and stable isotope data allows palaeoecological and palaeohydrological reconstructions, but ecological facies cannot be taken as direct stratigraphic or palaeoclimatic indices. There is no clear synchronism between either the salinity maxima or the freshwater stages observed in the different basins. For instance, marine-like conditions occurred at about 5,400 BP at Daïet el Melah, at which time rather dilute waters occupied the Hassi el Mejna basin. The reason for these discrepancies is that environmental status is here controlled by the local water and ion balances (ratios between input, evaporation storage and seepage) rather than by climatic fluctuations which would imply synchronous changes at the regional scale.

For approximately 6,000 yr (9,300–3,000 yr BP), sporadic supplies of dilute solutions occurred in these basins in the form of groundwater floods generated by local rain and/or wadi under-

flows. The freshwater inputs underwent various stages of evaporative concentration.

These water supplies document the occurrence of a general humid episode in the northern Sahara during early and middle Holocene^{16–18}. After 3,000 yr BP, groundwater fell to its present level as a consequence of overall climatic desiccation.

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Fossil packrat middens and the tandem accelerator mass spectrometer

Thomas R. Van Devender*, Paul S. Martin†, Robert S. Thompson‡, Kenneth L. Cole§, A. J. Timothy Jull||, Austin Long†, Laurence J. Toolin|| & Douglas J. Donahue||

* Arizona-Sonora Desert Museum, Route 9, Box 900, Tucson, Arizona 85743, USA

† Department of Geosciences, University of Arizona, Tucson, Arizona 85721, USA

‡ Department of Geological Sciences, Brown University, Providence, Rhode Island 02912, USA

§ Indiana Dunes National Lakeshore, 1100 N. Mineral Springs Rd, Porter, Indiana 46304, USA

|| National Science Foundation Facility for Radioisotope Analysis, University of Arizona, Tucson, Arizona 85721, USA

Analyses of well-preserved plant remains from ancient packrat (*Neotoma*) middens have yielded much information on the history of vegetation, fauna and climate from the more arid portions of North America over the past 40,000 years^{1,2}. In most of the modern deserts, woodland or forest communities were present during the last glacial age, the Wisconsin. Radiocarbon dating of packrat midden assemblages from a single cave or from several nearby sites in a homogeneous local area can yield a detailed, local vegetation chronology for many thousands of years from within 30–50 m on the rocky slopes in front of the dry rock shelters^{3,4}. The development of radiocarbon dating using the tandem accelerator mass spectrometer (TAMS) allows direct dating of very small samples. Here we present 20 examples of TAMS radiocarbon dates on packrat midden materials to illustrate their usefulness in testing anomalous mixtures of species in Ice Age communities, understanding the details of plant migrations, and refining Wisconsin and Holocene time boundaries based on range changes of important plant species (Table 1, Fig. 1).

Table 1 Accelerator and standard radiocarbon dates on packrat midden fossils

Sample	County, State*	Elevation (m)	Laboratory TAMS	No. standard	Material dated	Date (yr BP)
Contamination by younger material						
1. Ladder Cave 2B	White Pine, NV	2,060	AA-776†	A-2092	<i>Rhus trilobata</i> seeds, twigs	8,810 ± 420
						17,960 ± 1,100
2. Nankowep 9D	Coconino, AZ	2,060	AA-765†	A-1965	<i>Agave utahensis</i> seeds	9,520 ± 360
					<i>Pinus flexilis</i> needles	23,385 ± 770
3. Shafter 1B	Presidio, TX	1,310	AA-430†	A-1581	<i>Castela stewarti</i> seed	11,460 ± 420
					<i>Juniperus</i> sp. twigs	15,695 ± 230
4. Navar Ranch 5	El Paso, TX	1,340	AA-381†	A-2934	<i>Fouquieria splendens</i> thorns	4,200 ± 600
					<i>Quercus pungens</i> acorns	7,950 ± 330
5. Castle Mts 2C	Pima, AZ	790	AA-773†	A-2016	<i>Carnegiea gigantea</i> seeds	5,630 ± 470
					<i>Juniperus</i> sp. twigs	25,210 ± 1,000
Contamination by older material						
6. Redtail Peaks 1	San Bernardino, CA	520	AA-380†	A-1668	<i>Pinus monophylla</i> needles	11,360 ± 500
					<i>Juniperus californica</i> twigs	9,160 ± 170
				A-1580	<i>Juniperus californica</i> twigs	8,910 ± 380
7. Ernst Tinaja 1	Brewster, TX	810	AA-771†	A-3125	<i>Pinus remota</i> needles	17,900 ± 800
					Midden debris	7,820 ± 220
				A-2964	<i>Neotoma</i> faecal pellets	8,560 ± 380
8. Tinajas Altas 16A	Yuma, AZ	380	AA-780†	A-3521	<i>Juniperus californica</i> twigs	18,530 ± 1,070
					<i>Neotoma</i> faecal pellets	7,860 ± 100
Animal remains						
9. Quade	Clark NV	1,060	AA-782	None	<i>Ochotona</i> pellets	14,300 ± 800
Other small samples						
10. Tank Trap Wash 1:1	El Paso, TX	1,340	AA-1162		<i>Juniperus</i> sp. seeds	42,000 ± 3,600
						-2,500
				A-1723	<i>Juniperus</i> sp. twigs	>35,000
11. Brass Cap Point 2	Yuma, AZ	550	AA-574	None	<i>Castela emoryi</i> seed	9,750 ± 470
12. Tinajas Altas 15A(L)	Yuma, AZ	550	AA-452	None	<i>Pinus monophylla</i> needles	15,680 ± 700
13. Butler Mts. 3B	Yuma, AZ	240	AA-770	None	<i>Juniperus californica</i> seeds	11,060 ± 300
14. Butler Mts. 3A	Yuma, AZ	240	AA-769	None	<i>Larrea divaricata</i> twigs	11,250 ± 410
15. Navar Ranch 1BO	El Paso, TX	1,430	AA-772		<i>Bouteloua curtipendula</i> spikelets	>30,000
				A-1644	<i>Juniperus</i> sp. twigs	>34,000
16. Baby Vulture Den 5B(2)	Brewster, TX	680	AA-382‡	A-3133	<i>Prosopis glandulosa</i> leaflets	21,300 ± 1,250
					<i>Juniperus</i> sp. twigs	19,600 ± 800
17. Tinajas Altas 3B	Yuma, AZ	580	AA-781‡	USGS-959	<i>Ambrosia dumosa</i> leaves	10,600 ± 420
					<i>Neotoma</i> faecal pellets	10,300 ± 110
18. Tinajas Altas 16	Yuma, AZ	580	AA-779‡	A-3521	<i>Cercidium floridum</i> twig	8,650 ± 430
					<i>Neotoma</i> faecal pellets	7,860 ± 100
19. Tinajas Altas 13B	Yuma, AZ	580	AA-777‡	A-3507	<i>Bursera microphylla</i> seeds	5,820 ± 310
					<i>Neotoma</i> faecal pellets	5,940 ± 70
20. Alamo Canyon 1U	Pima, AZ	915	AA-533‡	A-2211	<i>Carnegiea gigantea</i> seeds	9,230 ± 370
					<i>Quercus ajoensis</i> acorns	9,910 ± 210

* NV, Nevada; AZ, Arizona; TX, Texas. † Not within 2 s.d. of standard date. ‡ Within 2 s.d. of standard date.

In the absence of a continuous stratigraphic record, midden analysts depend on radiocarbon dating for chronological control. Conventional radioactive decay counters typically require 2–10 g of material for carbon dioxide gas dates and 10–20 g for benzene liquid scintillation samples. About 900 standard radiocarbon determinations have been obtained from packrat middens⁵. In contrast, TAMS dates measure the actual number of ¹⁴C atoms, rather than rates of radioactive decay, in samples as small as 10 mg (refs 6, 7). With TAMS dating, most of the specimens of ecologically important species can be saved for other purposes such as radioisotope analyses of deuterium and ¹⁸O as indicators of palaeoclimates. The TAMS method also allows direct dating of small Middle Wisconsin samples when the 10 g of sample needed for a high-precision standard date is not available. For example, most of the juniper (4.7 g) in a midden from the Hueco Mountains of Texas yielded a standard date of >34 kyr (thousands of years before 1950) while a few remaining seeds gave a finite TAMS date of 42 kyr (AA-1162).

Many of the standard dates on midden materials were obtained on combined specimens (rarely was there a large single specimen) of individual plant species of biogeographical interest after they had been disaggregated in water^{8,9}. In another method, unwashed pieces of midden matrix were dated after the plants on its surface had been identified¹⁰. The assumption of contemporaneity of the plants in the assemblages is reasonable in both procedures if the sample was collected from a discrete stratigraphical unit, if any outer weathering rind was removed, and if the ancient packrats were not fossil collectors themselves. Plant fossils of different species found together in a single midden sample that do not agree with current hypotheses of

community composition are especially likely to generate persistent questions about sample integrity¹¹. TAMS radiocarbon dates on small samples (combined or single specimens) can verify that the age of a species of particular biogeographical interest is not significantly different in age from an associated standard date or dates on the midden.

In several cases the standard dates were not verified by TAMS dates. In the Snake Range of Nevada, skunkbush (*Rhus trilobata*; 8.8 kyr, AA-776) proved to be a contaminant in a much older deposit containing Great Basin bristlecone pine (*Pinus longaeva*)¹². In the Grand Canyon of Arizona, a Utah agave (*Agave utahensis*; 9.5 kyr, AA-765) leaf proved to be 13.9 kyr younger than limber pine (*Pinus flexilis*) in the sample¹³. In these examples, apparent anomalous associations in glacial-age mixed-conifer forest assemblages were disproven. In the Livingston Hills (Chinati Mountains) of Trans-Pecos, Texas, seeds of Chihuahuan crucifixion thorn (*Castela stewarti*) were 4,000 yr younger than the full-glacial standard date (15.7 kyr, A-1581)¹⁴. The presence of this low desert shrub in a pinyon-juniper-oak woodland, an anomalous association by current standards, remains likely because of the Late Wisconsin age (11.5 kyr, AA-430)¹⁵. Interestingly, a TAMS date of 9.8 kyr (AA-574) on the closely related Sonoran crucifixion thorn (*Castela emoryi*) establishes its presence in an Early Holocene juniper-Joshua tree (*Yucca brevifolia*) – Bigelow beargrass (*Nolina bigelovii*) woodland/chaparral community which presently occurs much higher in elevation than the present Sonoran desert-scrub habitat of *C. emoryi*. Although the stem of ocotillo or coachwhip (*Fouquieria splendens*), a characteristic shrub of the Chihuahuan Desert, proved to be a contaminant in an Early

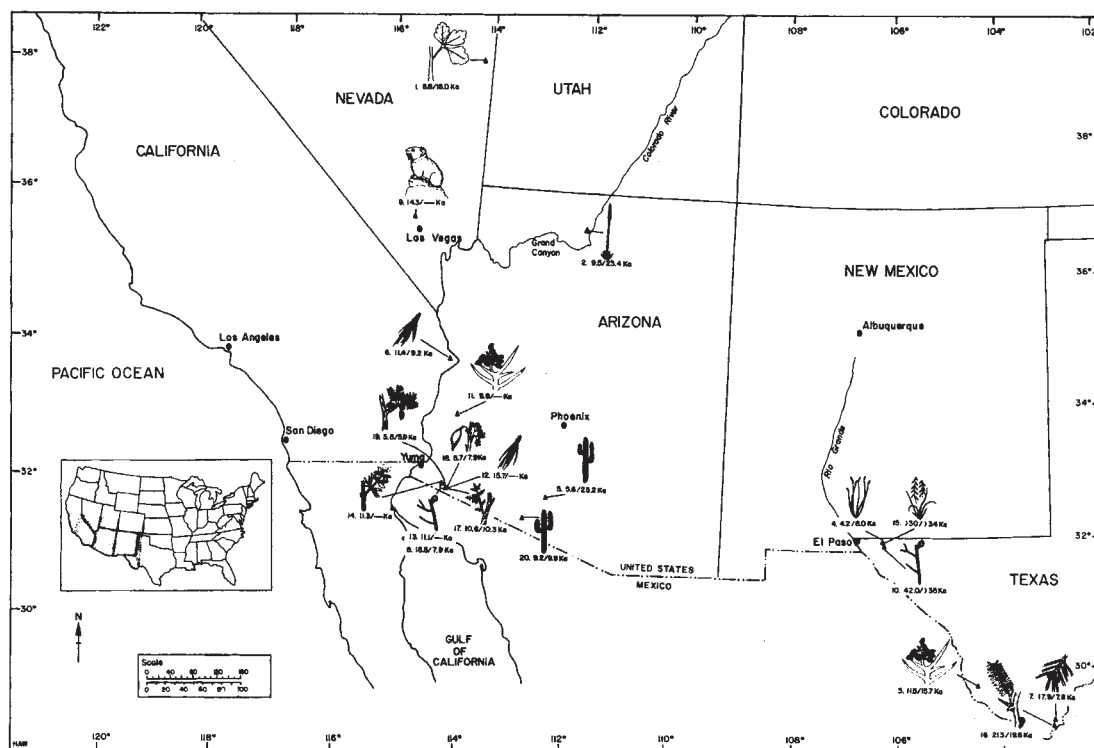


Fig. 1 Map of the southwestern United States, illustrating plant and animal remains from packrat middens radiocarbon-dated using tandem accelerator mass spectrometer. Numbers match Table 1; dates are TAMS/standard kyr (ka).

Holocene assemblage, the TAMS date records its arrival in the Hueco Mountains, Texas by Middle Holocene (4.2 kyr, AA-381).

Radiocarbon dating of older contaminants in packrat assemblages is especially useful in refining bioclimatic time boundaries which are usually based on the range shifts of climatically sensitive species in a chronological sequence. In much of the southwestern United States a retreat of pinyon pines from desert lowlands at about 11,000 yr ago marks the end of the Wisconsin glacial age^{1,15}. Dubious Holocene survivals of single-leaf pinyon (*Pinus monophylla*) in the Whipple Mountains of California¹⁶⁻¹⁸ and of papershell pinyon (*Pinus remota*) in the Big Bend of Texas¹⁹ were rejected when their needles yielded Wisconsin ages (11.4 kyr, AA-380, and 17.9 kyr, AA-771). A lingering survival of California juniper (*Juniperus californica*) in the Sonoran Desert lowlands of the Tinajas Altas Mountains of southwestern Arizona, suspected on the basis of a few twigs found in an Early Holocene midden, was not supported by the TAMS measurement (18.5 kyr, AA-780)²⁰. Note that all of the midden contaminants were suspected before TAMS dating and represent an extremely low percentage of the total number of specimens in the midden assemblages.

The ability to date very small samples has several special applications. Tickseed (*Corispermum*), a diminutive member of the salt bush family, was recently established as native to North America, and not a historical introduction, through TAMS dating of seeds from a western Grand Canyon midden²¹. Faecal pellets of pika (*Ochotona cf. princeps*), from near Corn Springs at 1,060 m elevation in the Las Vegas Valley of southern Nevada, were associated with remains of a Late Wisconsin juniper woodland (14.3 kyr, AA-782, Fig. 2). We believe that pikas are inhabitants of alpine meadows and openings in montane forests and do not currently occur in the low-elevation xeric woodlands²². The TAMS dates on single-leaf pinyon needles (15.7 kyr, AA-452; Fig. 2) at 550 m elevation in the Tinajas Altas Mountains and on California juniper seeds (11.1 kyr, AA-770) at 240 m in the Butler Mountains, both in southwestern Arizona, provide direct dates on uncommon woodland plant remains at their Late Wisconsin lower elevational limits.

TAMS dates are especially useful in verifying the presence of desert or grassland plants in Late Wisconsin or Early Holocene woodlands. In the Butler Mountains, an accelerator date of 11.3 kyr (AA-769) on creosote bush (*Larrea divaricata*)

indicates its co-occurrence with California juniper (AA-770, cited above) south and east of regions where their modern ranges overlap. AA-769 is the first pre-Holocene (>11,000 yr BP) date establishing the presence of creosote bush, an important dominant in the warm deserts of North America, within the lowest elevations of its present range in the United States. In the Hueco Mountains, a TAMS date on spikelets of side-oats grama (*Bouteloua curtipendula*, Fig. 2) revealed that this wide-ranging grass was present in Middle Wisconsin pinyon-juniper-oak woodland over 30 kyr (AA-772). Side-oats grama is an important C_4 perennial in much of the summer rainfall grasslands of the southwestern United States. For the first time, grasses, which are potentially excellent palaeoenvironmental indicators, can be dated directly. At Rio Grande Village in Big Bend National Park, honey mesquite (*Prosopis glandulosa*) persisted in a full-glacial pinyon-juniper-oak community about 900 m lower than similar modern associations²². Honey mesquite is presently an important dominant in Chihuahuan desert-grassland communities.

Holocene middens from the Tinajas Altas Mountains of southwestern Arizona record the arrivals of characteristic Sonoran Desert species, including white bursage (*Ambrosia dumosa*) by 10.6 kyr (AA-781), blue palo verde (*Cercidium floridum*) by 8.7 kyr (AA-779) and elephant tree (*Bursera microphylla*) by 5.8 kyr (AA-777). The latter, a tropical genus at its northern limit, is especially frost sensitive. Some time in the Late Holocene, foothills palo verde (*Cercidium microphyllum*) arrived to replace blue palo verde, which is now confined at low elevations to flood plains, arroyo banks and roadsides.

In the Ajo Mountains of Arizona, a few saguaro (*Carnegiea gigantea*) seeds from a midden yielded an age of 9.2 kyr (AA-533). Saguaro is the stately, frost-sensitive, arborescent cactus that is the hallmark of the Arizona Upland subdivision of the Sonoran Desert²³. The TAMS results represent a minimum age for arrival of saguaros in Organ Pipe Cactus National Monument. Further east (75 km north-east) in the Castle Mountains, a midden dated on juniper by standard methods at 25.2 kyr offered the possibility of obtaining an unusually old (glacial age) record on associated seeds of saguaro (Fig. 2). Although the result of 5.6 kyr (AA-773) reveals contamination, it shows that the giant cacti were present and available as a food resource for Middle Holocene gathering and hunting people in the region

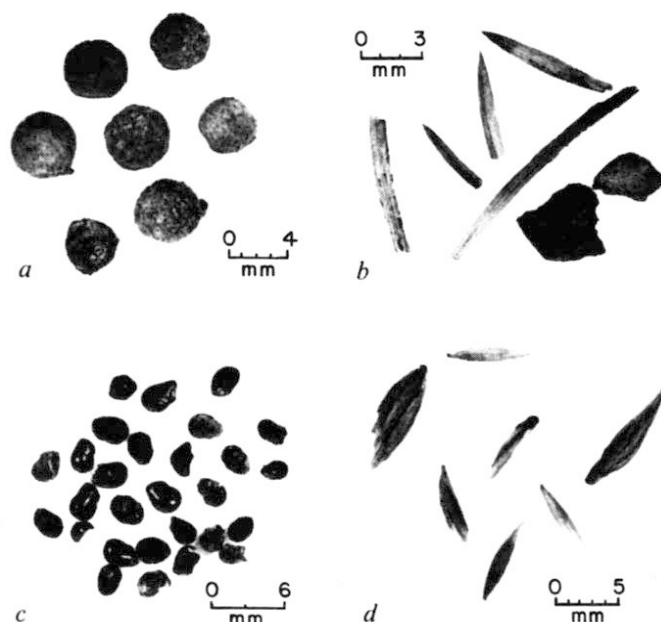


Fig. 2 Packrat midden specimens dated by TAMS. *a*, Faecal pellets of pika from Quade, Las Vegas Valley, Nevada (14.3 kyr, AA-782). *b*, Single-leaf pinyon needles and nut fragments from Tinajas Altas 15A(L), Arizona (15.7 kyr, AA-452). *c*, Saguaro seeds from Alamo Canyon 1(U), Ajo Mountains, Arizona (9.2 kyr, AA-533). *d*, side-oats grama spikelets from Navar Ranch 1B, Hueco Mountains, Texas (>30 kyr, AA-772).

of Ventana Cave, one of the best-known archaeological sites in the Sonoran Desert²⁴.

In the process of dating plant fragments of particular biogeographical interest, it was possible to replicate standard dates previously run on middens that were the source of the TAMS sample (for example, AA-533 with A-2211, AA-781 with USGS-959, AA-777 with A-3507). Tests confirming both dating methods can be matched with other cases in which suspected redeposition or mixing of fossils in the deposit was verified (Table 1). The TAMS results sustained our ecological bias that neither skunk-bush (AA-776) nor Utah agave (AA-765) grew with sub-alpine conifers, and that single-leaf pinyon (AA-380) and papershell pinyon (AA-771) did not occur in dry Early Holocene woodlands in California and Texas, as suggested by a few midden specimens. The middens indicate that in the Late Wisconsin (AA-775), pikas lived in juniper woodland, a habitat now foreign to them. TAMS dates (AA-430, AA-574) on Chihuahuan and Sonoran crucifixion thorns imply that these spiny desert shrubs were living in late-glacial pinyon-juniper-oak and Early Holocene juniper-Joshua tree woodlands, respectively, but survive in hot desert scrub habitats at lower elevations today. TAMS dates will be critical in distinguishing between cases of sample contamination or redeposition and cases of authentic anomaly when future unexpected mixtures are found.

Our findings indicate that by 11,000 yr ago at least, creosote bush (AA-769), the common desert shrub, occupied the southern part of its present range in the United States. In southwestern Arizona, important Sonoran Desert succulents, trees and shrubs including saguaro (AA-533), white bursage (AA-781), blue palo verde (AA-779) and elephant tree (AA-777), appeared in the Holocene, with foothills palo verde (no direct date) arriving last. Fossils of frost-sensitive warm desert species have not been discovered during the height of the last glacial maximum (22,000 to 14,000 yr BP), a time when pinyon (AA-452), oak, juniper (AA-780), Joshua tree, sagebrush and other temperate woodland or cool desert shrubs occupied what is now warm desert lowlands^{1,2}. Mixed conifer forests with limber pine (AA-534) moved into higher elevations in the Grand Canyon.

Through the method of accelerator dating of plant parts from ancient packrat middens, it is now possible to locate glacial-age

biotic refugia, to determine the timing of plant migrations into arid parts of the western United States since the last ice age, and to detect any unusual associations of species that are anomalous by modern standards. Historical ecologists and biogeographers have gained a powerful new technique.

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Global patterns of soil nitrogen storage

Wilfred M. Post & John Pastor

Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, USA

Paul J. Zinke & Alan G. Stangenberger

Department of Forestry and Resource Management, University of California, Berkeley, California 94720, USA

The amount of nitrogen stored in soil is related to climate through biotic processes associated with productivity of vegetation and decomposition of organic matter. Other factors, particularly rainfall input, dry deposition input, nitrogen fixation and losses of inorganic nitrogen due to leaching contribute to the variability of nitrogen storage. Here we report that soil nitrogen storage ranges from 0.2 kg m⁻³ in warm deserts to 2 kg m⁻³ in rain tundra, with a peak of 1.6 kg m⁻³ in subtropical wet forests. Soil carbon storage shows a similar pattern. The global nitrogen pool in the surface metre of soil comprises an estimated 9.5 × 10¹³ kg. Each soil profile

examined was classified according to the Holdridge life zone¹ in which it was found. Soil carbon/nitrogen ratios range from <10 in tropical deserts to >20 in cool, wet forests or rain forests. We determined C/N ratios of 15–20 in cool life zones and 10–15 in warm life zones. These results indicate that: (1) relatively large amounts of soil nitrogen in wet tropical regions are associated with recalcitrant humic materials in an advanced state of decay, with low C/N ratios; (2) the seasonal climate contrast in temperate regions, combined with variable litter quality due to the mix of coniferous and deciduous species, results in moderate carbon and nitrogen storage in soil and variable C/N ratios; and (3) slow decomposition in wet tundra regions results in high carbon and nitrogen storage, with high C/N ratios.

The present analysis of global soil nitrogen pools is based on data from 3,100 soil profiles supporting natural vegetation (excluding wetlands), therefore the patterns discussed do not account for gains or losses caused by man's activities. The data were obtained from P.J.Z. and from soil survey literature. Details of sample collection chemical analyses, data computation and data sources are reported elsewhere².

Samples from each soil profile were collected from each horizon or at standard depths (0, 5, 10, 20, 40, 60, 80 and 100 cm). Carbon was analysed using dry combustion and total nitrogen was determined by Kjeldahl digestion. Soil carbon and nitrogen masses were calculated by multiplying the per cent of each by the bulk density obtained for each layer, and the horizon thickness. Data from the literature were used if available for the complete soil profile, or to a depth of 1 m. If literature sources did not report bulk density, this parameter was estimated using a regression formula incorporating depth of the profile, organic carbon content and vegetation type (see ref. 2).

The Holdridge life-zone system, applied here, is an objective classification based on climatic data (Fig. 1a). Previous work has demonstrated its use in relating the key soil-forming factors of vegetation and climate^{3–6} as well as in examining the relationship between soil carbon and climate⁷. A Holdridge life zone is defined by the climate variables biotemperature and annual precipitation, where biotemperature is the annual mean unit-period temperature with substitution of 0 °C for all temperatures

<0 °C and >30 °C. A third variable, the potential evapotranspiration/precipitation ratio, is correlated with precipitation and biotemperature and appears as the third axis in Fig. 1. Whenever possible, the climate variables were calculated from meteorological data for each profile. If adequate meteorological information for direct classification of the profile was not available, associated vegetation information was used to classify the soil profiles.

Summary statistics were computed for each life zone class (Table 1). Because most soil nitrogen is organically bound, nitrogen storage in soil shows a pattern similar to that of organic carbon storage. However, the nitrogen storage patterns across various life zones differ in detail from carbon storage patterns (Fig. 1a, b). Detailed patterns in carbon storage are related to the potential evapotranspiration/precipitation ratio. Where this ratio is 1, carbon storage is 10 kg m⁻³ and decreases as evapotranspiration exceeds precipitation and increases as the latter exceeds the former. This carbon storage pattern is modified by low storage in dry subtropical and warm temperate deciduous forests with strong seasonal contrast. Nitrogen storage has a component that is strongly influenced by precipitation, perhaps caused by direct inputs and leaching losses, particularly in cool temperate and boreal forest life zones with lower nitrogen storage than expected from considering evapotranspiration alone.

We can infer considerable information concerning soil nitrogen dynamics by examining soil C/N ratios (Fig. 1c). Litter has a higher C/N ratio than the humus derived from it. As decomposition proceeds, easily decomposed material disappears and nitrogen is immobilized in microbial biomass and decay products, leaving behind more recalcitrant material with slower decomposition rates and lower C/N ratios. Factors slowing the decay rate, such as high lignin/nitrogen ratios, cool temperatures and poor soil aeration^{8–11}, will retard this decrease in the C/N ratio. Conversely, one can expect lower soil C/N ratios with increasing decomposition rate. As humus decomposes and its C/N ratio decreases, it is being incorporated into the soil profile. The balance between the rate of decomposition and the rate of incorporation into the soil profile, both functions of climate, will determine the C/N ratio of the soil humus.

Table 1 Organic carbon and nitrogen storage, and C/N ratio by Holdridge life zone

Life zone*	No. of samples	Carbon (kg m ⁻³)		Nitrogen (g m ⁻³)		C/N ratio	
		Mean	s.d.	Mean	s.d.	Mean	s.d.
Dry tundra	5	3.1	2.2	—	—	—	—
Moist tundra	12	10.9	6.4	638.5	231.7	18.3	6.5
Wet tundra	33	20.7	16.0	1,251.3	958.1	18.4	6.4
Rain tundra	8	36.6	12.9	2,226.0	593.6	15.6	2.8
Boreal dry bush	8	10.2	4.6	631.0	239.6	16.0	3.4
Boreal moist forest	305	15.5	30.0	1,034.1	1,091.5	16.0	8.4
Boreal wet forest	24	15.0	10.7	980.1	777.5	16.9	5.7
Boreal rain forest	23	32.2	35.2	1,512.2	1,633.7	25.8	11.6
Cool temperate desert	4	9.7	0.7	—	—	—	—
Cool temperate desert bush	129	9.9	6.0	779.8	445.0	12.5	3.7
Cool temperate steppe	375	13.3	9.5	1,032.2	737.5	15.1	8.8
Cool temperate moist forest	650	12.0	8.2	626.1	494.7	22.5	8.7
Cool temperate wet forest	442	17.5	23.8	930.6	1,161.6	20.7	8.4
Cool temperate rainforest	17	20.3	12.7	1,210.3	441.9	15.7	4.6
Warm temperate desert	9	1.4	1.0	106.5	84.5	15.3	4.6
Warm temperate thorn steppe	291	7.6	6.8	538.0	484.7	15.9	10.1
Warm temperate dry forest	105	8.3	4.6	644.8	331.2	14.0	6.7
Warm temperate moist forest	98	9.3	7.4	648.3	608.0	25.1	13.1
Warm temperate wet forest	52	26.8	13.4	2,806.6	806.4	15.6	6.4
Subtropical desert bush	1	29.1	—	2,282.6	—	12.7	—
Subtropical thorn woodland	18	5.4	2.2	379.3	104.8	14.3	3.0
Subtropical dry forest	17	11.5	13.9	1,070.4	1,035.9	10.2	4.3
Subtropical moist forest	14	9.2	4.5	987.9	581.9	10.3	3.8
Subtropical wet forest	1	9.4	—	2,853.4	—	3.3	—
Tropical thorn woodland	2	2.6	2.1	264.6	184.8	9.2	1.3
Tropical very dry forest	124	6.9	4.9	597.2	730.8	13.7	5.2
Tropical dry forest	184	10.2	11.1	885.9	579.1	13.3	16.6
Tropical moist forest	163	11.4	11.7	802.9	546.1	14.9	8.6
Tropical wet forest	23	15.0	9.2	655.2	229.1	30.2	12.8
Total	3,137						

* We have simplified the life-zone classification by combining the altitudinal classes with the appropriate latitudinal classes. For example, lower montane tropical dry forest is combined with warm temperate dry forest. Life zones for which we have no data have been omitted. 15% of the profiles do not have nitrogen measurements corresponding to the carbon measurements.

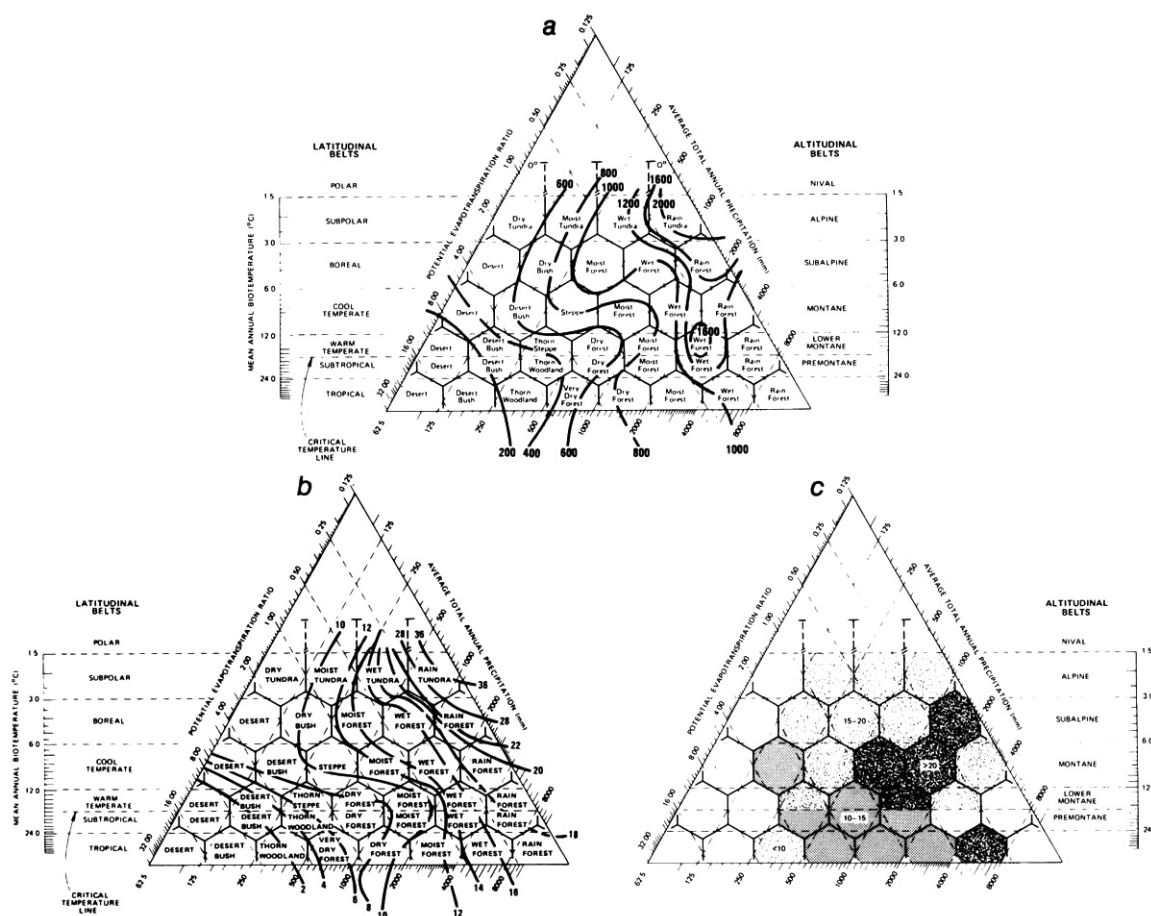


Fig. 1 a, Soil nitrogen storage (g m^{-3}) isopleths on the Holdridge life-zone chart. b, Soil carbon storage (kg m^{-3}) isopleths on the Holdridge life zone chart. c, C/N ratio by Holdridge life zone. Shading indicates range of mean C/N ratios for life zones. Those life zones for which we have no data are blank.

In boreal and tundra life zones, decomposition rates are low because of low temperatures, low litter quality, and partially water-saturated soils. In some tundra regions, the decomposition rate is slower than the production rate, resulting in net accumulation of organic matter¹². The soils of these regions have a high C/N ratio, as well as high carbon and nitrogen storage.

In cool and warm temperate life zones, decomposition is, in the long term, in balance with production, leading to steady-state humus levels in the soil under undisturbed vegetation. Soil humus C/N ratios in these life zones are highly variable, ranging from 10 to >20 , and are somewhat higher in forests in higher latitudes². Some of this variation is the result of litter quality. Mean C/N ratios of litterfall in the forest life zones of these regions range from 45 to 65 (refs 13–16) depending on species composition. Life zones of higher latitudes contain a larger fraction of coniferous species with litter high in lignin^{9–11} and relatively low in nitrogen. There is also a decrease in the C/N ratio of litterfall with decreasing latitude^{13–17}.

Litter production in moist and wet tropical and subtropical life zones is high^{16,17}. However, most of this litter is decomposed relatively rapidly compared with that in more poleward life zones^{18,19}; this is largely because of year-round temperature and moisture being favourable for decomposition. As a result, the C/N ratio of the organic matter rapidly approaches a low value, ultimately limited by the C/N ratio of slowly decomposing recalcitrant materials and microbial detritus incorporated into the soil.

The lowest C/N ratios occur in the warmest life zones. Where precipitation is high (in tropical forests), low C/N ratios are caused by an advanced state of organic matter decay. By contrast, in deserts where precipitation is low, the available soil moisture limits both net primary productivity and leaching,

resulting in low nitrogen uptake by plants, low carbon inputs, an accumulation of inorganic nitrogen salts in the soil, and a low C/N ratio²⁰.

Annual amounts of nitrogen in litterfall, averaging $2\text{--}13 \text{ g m}^{-2} \text{ yr}^{-1}$ depending on life zone^{13–17}, are larger than the average fluxes because of nitrogen fixation, precipitation input and leaching. Rates of atmospheric nitrogen input are estimated to range from $1 \text{ to } 10 \times 10^{10} \text{ kg yr}^{-1}$ globally, or an average of $0.08\text{--}0.76 \text{ g m}^{-2} \text{ yr}^{-1}$ (ref. 21). Biological nitrogen fixation for all non-agricultural land is $\sim 5 \times 10^{10} \text{ kg yr}^{-1}$, or $0.4 \text{ g m}^{-2} \text{ yr}^{-1}$ (ref. 22). Nitrogen is lost from soils through denitrification and leaching. Denitrification, performed by facultative anaerobes, varies in time and space, depending on the supply of nitrate and the anaerobic condition of the soil. Rates of denitrification are likely to be small except in wetlands. Leaching losses may be important in some regions. An upper bound on the global impact of leaching can be estimated from the flux of dissolved nitrogen in rivers and of particulate organic nitrogen in the case of seasonally flooded ecosystems. The total nitrogen lost to rivers is $3.1 \times 10^{10} \text{ kg yr}^{-1}$, averaging $0.25 \text{ g m}^{-2} \text{ yr}^{-1}$ of land surface, although a large fraction of this nitrogen may be derived from disturbed agricultural land²³.

We conclude that ecosystem nitrogen cycling and litter quality, both of which are climatically influenced, determine the patterns of soil nitrogen storage. In addition, availability of phosphorus²⁴ as well as other soil-forming factors²⁵, or the role of consumers in processing and transporting nitrogen²⁶, may contribute to the large variability of the mean nitrogen storage in soils of climatically defined life zones.

Estimates of the global total of soil organic nitrogen range from $7 \times 10^{13} \text{ kg}$ (ref. 27) to $8.2 \times 10^{14} \text{ kg}$ (refs 28, 29), with a few intermediate estimates of $1.7 \times 10^{14} \text{ kg}$ (ref. 23), $7.6 \times 10^{14} \text{ kg}$ (ref. 30), $1.75 \times 10^{14} \text{ kg}$ (refs 31, 32), and $3 \times 10^{14} \text{ kg}$ (refs 21, 33).

Table 2 Areas of global climates (Holdridge life zones) and estimated carbon and nitrogen content in soil profiles to 1-m depth

Life zone*	Area ($\times 10^{12}$ m ²)	Carbon content ($\times 10^{15}$ g)	Nitrogen content ($\times 10^{12}$ g)	Life zone*	Area ($\times 10^{12}$ m ²)	Carbon content ($\times 10^{15}$ g)	Nitrogen content ($\times 10^{12}$ g)
Ice†	2.071	0	0	Warm temperate moist forest	7.801	72.6	5,062.6
Dry tundra	0	0	0	Warm temperate wet forest	0.570	15.3	1,029.8
Moist tundra	2.701	29.4	1,724.6	Warm, temperate rain-forest	0.029	0.8	20.3
Wet tundra	1.497	31.0	1,873.2	Subtropical desert	1.385	1.9	138.5
Rain tundra	0.030	1.1	66.8	Subtropical desert bush	1.535	4.6	3,503.8
Boreal desert	0.022	0.2	11.0	Subtropical thorn woodland	1.651	8.9	626.2
Boreal dry bush	1.282	13.1	808.9	Subtropical dry forest	3.500	40.3	3,746.4
Boreal moist forest	12.722	197.2	13,155.8	Subtropical moist forest	7.985	73.5	7,888.4
Boreal wet forest	4.350	65.3	4,263.4	Subtropical wet forest	0.499	4.7	1,423.8
Boreal rainforest	0.303	9.8	458.2	Subtropical rainforest	0.014	0.2	8.4
Cool temperate desert	1.241	12.0	620.5	Tropical desert	8.420	8.4	421.0
Cool temperate desert bush	3.571	35.4	2,784.7	Tropical desert bush	2.321	2.3	116.1
Cool temperate steppe	9.045	120.3	9,336.2	Tropical thorn woodland	2.351	6.1	622.1
Cool temperate moist forest	9.344	112.1	5,850.3	Tropical very dry forest	4.712	32.5	2,814.0
Cool temperate wet forest	1.626	28.5	1,513.2	Tropical dry forest	10.018	102.2	8,874.9
Cool temperate rain-forest	0.256	5.2	204.8	Tropical moist forest	8.605	98.1	6,909.0
Warm temperate desert	1.911	2.7	203.5	Tropical wet forest	0.378	5.5	247.7
Warm temperate desert bush	4.894	29.4	1,468.2	Tropical rainforest	0.003	0.1	1.8
Warm temperate thorn steppe	5.171	39.3	2,782.0	Total	131.336	1,272.4	95,433.7
Warm temperate dry forest	7.543	62.6	4,863.7				

* See Table 1 legend.

† Does not include Greenland.

All of these estimates were obtained by dividing values for organic carbon storage by the C/N ratio. We present below the first estimate of the global storage of organic nitrogen derived from direct measurements.

A map of Holdridge life zones, based on the average climate from 1930 to 1970, has recently been produced for the globe⁶. This map allows the calculation of the areal extent of Holdridge life zones, which, when combined with the results of Fig. 1, can be used to compute a global estimate of organic soil nitrogen (Table 2), assuming all vegetation is in equilibrium with this average climate (that is, potential natural vegetation). Table 2 includes a new estimate of organic soil carbon that incorporates more profile data and different areas of life zones than our previous report⁷. The global soil carbon value of 1.272×10^{15} kg is well within one standard deviation of our previous estimate of 1.395×10^{15} kg. Our global estimate for soil nitrogen is 9.5×10^{13} kg. To represent present-day actual soil nitrogen storage, this value should be lowered to account for losses due to agricul-

ture. The global size of such losses, however, is presently unknown.

If we assume that fixation and denitrification rates are negligible in the soils of our database; that plant uptake and litter return are in steady state; and that the 3.1×10^{10} kg yr⁻¹ of nitrogen lost annually to rivers²⁷ represents the net nitrogen output from the global soil pool, the turnover rate for the global soil nitrogen pool is of the order of 3,000 yr. Relaxing the above assumptions should decrease this value.

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A paternal influence on survival of wild mice in the nest

S. A. Barnett & R. G. Dickson

Department of Zoology, Australian National University,
GPO Box 4, ACT, 2601 Australia

For an infant mammal in the nest, the principal environmental variables are those provided by the parents. A series of studies has been made on parental influences on the development of house mice, especially in relation to cold adaptation^{1,2}. During this work the role of the father has usually been ignored. Here we present evidence that such inverted sexism is unjustified. Mated pairs, of which the male was from the tenth generation of a stock of wild-type mice kept in a cold environment, reared a higher proportion of their young than did pairs of which the male was from a control stock. The influence of the male on variance in survival in the nest was greater than that of the female.

Two stocks of wild-type house mice, *Mus musculus*, were used, derived from mice trapped in the Australian Capital Territory. Each stock was a closed colony, one maintained at 23 °C (controls), the other at 3 °C ('Eskimo' mice). Pairs were each kept in plastic cages with wire lids, and were permanently mated. Members of a pair were never siblings. Rat cubes and water were freely available. Bedding consisted of wood shavings to cover the cage floor, and ~5 g of non-absorbent cotton wool.

Changes observed over generations have been reported elsewhere³. In the cold environment, during early generations, many pairs were barren, and there was a high nestling mortality. But fertility improved and, after 10 generations, the number of young weaned by an eskimo pair in the cold approached that of controls in the warm; and the eskimo mice were heavier than controls at all ages.

In the present study, at the ninth generation, 15 control pairs were transferred to the cold at mating, and 15 eskimo pairs were also mated. The young of these pairs were used in the experiments. They provided 60 pairs, 15 of each of the four possible kinds of mating, all in the cold environment: control ♂ × control ♀; control ♂ × eskimo ♀; eskimo ♂ × control ♀; eskimo ♂ × eskimo ♀. At mating, the mean body weight of the control females was 14.3 ± 0.4, and that of the eskimo females, 16.6 ± 0.4 g ($P < 0.001$, Student's *t*-test).

As expected, at 3 weeks the young of the eskimo mothers were heavier than those of control mothers, regardless of the type of father (Fig. 1). Differences in the number of young born to a pair were influenced by both parents (Table 1). When both members of a pair were controls, the mean number of young was about 18; the other three classes had means of 28 or above.

The most important finding, however, was on survival in the nest (Table 2). The two types of pairs with eskimo males reared

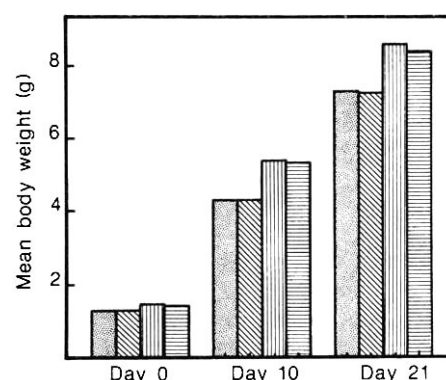


Fig. 1 Body weights of the offspring from the four types of mating. ■, Controls; ▨, hybrids with control mothers; ■, eskimo; ▩, hybrids with eskimo mothers.

a higher proportion of young than did the other two. The female had some influence; but, as the analysis of variance shows, the male had the greater effect. Mortality was due largely to losses of whole litters. Control males mated with control females lost 48% of their litters, and those mated with eskimo females, 39%; the corresponding figures for the eskimo × eskimo and eskimo × control pairs were 14% and 24%, respectively ($\chi^2 = 22.1$, d.f. = 3, $P < 0.001$).

In the present experiments, the factors that influenced growth in the nest differed from those that affected survival. A similar non-correspondence has been observed in a study of domestic mice in a cold environment⁴, but the physiological or behavioural mechanisms involved were not analysed.

Previous work, however, has some bearing on the parental efficiency of the eskimo mice. (1) Eskimo females secrete more concentrated milk than do control females in the same environment; this is evidently one source of the higher growth rate of eskimo young⁵. (2) For an explanation of the paternal influence on survival we must look to behaviour. There is evidence that the survival of young domestic mice in a cold environment is enhanced by parental attention, such as licking and carrying⁶. It is difficult to record parental behaviour in a closed nest, but eskimo mice have been shown to spend more time in parental activities than do control mice; in that study it was not possible to distinguish consistently between male and female parents: both attended to the young⁷.

Most experimental studies of the behaviour of male mice toward young have been on laboratory strains. Care of young occurs, but varies greatly with genotype and conditions⁸⁻¹⁰. Paternal care has also been recorded in other species of rodents¹¹. Present hypotheses concerning parental 'investment' imply that such species should be monogamous¹², yet most of the evidence suggests that in nature these small, polytocous

Table 1 Numbers of young born per pair

Male parent		Female parent		
		Control	Eskimo	
Control	No. of pairs	15	14	$P < 0.01$
	Mean ± s.e.	17.7 ± 2.2	32.4 ± 2.4	
Eskimo	No. of pairs	14	16	
	Mean ± s.e.	28.5 ± 2.6	28.0 ± 2.8	
		$P < 0.01$		

Analysis of variance

Source of variation	d.f.	Mean square	F	P
Female type	1	709	7.4	$P < 0.01$
Male type	1	140	1.5	$P < 0.3$
Interaction	1	857	8.9	$P < 0.005$
Residual	55	96		
Total	58	121		

Table 2 Numbers of young weaned by a pair (mean ± s.e.)

Male parent	Female parent		
	Control	Eskimo	
Control	9.0 ± 2.0 (48)	18.4 ± 3.4 (53)	P < 0.01
Eskimo	20.8 ± 3.2 (67)	23.5 ± 3.0 (81)	
	P < 0.01		

Analysis of variance for percentage of young weaned

Source of variation	d.f.	Mean square	F	P
Female type	1	1,339	1.8	$P < 0.2$
Male type	1	8,101	10.8	$P < 0.003$
Interaction	1	226	0.3	$P < 0.6$
Residual	55	747		
Total	58	881		

Mean per cent weaned is given in parentheses.

mammals are promiscuous or polygamous. Facultative monogamy is, however, a possibility¹³. It has not been reported in populations of house mice, but this widely dispersed species is well known to be ecologically exceptionally versatile^{14,15}. The house mouse is not only capable of thriving in a great variety of environments: studies of free-living populations suggest also that, in different cold climates, there are alternative genotypes and phenotypes, each suited to severe conditions¹⁶.

Our experiments on wild mice in captivity have enabled us to compare eskimo mice with controls in the same environment. The superior growth rate, fertility and other features of the cold-adapted mice have been observed in stocks derived from Scottish as well as Australian populations^{2,3}. Such comparisons, together with cross-fostering¹⁷, have shown that cold-adaptation depends on a combination of direct ontogenetic responses, genetical changes and also alterations in maternal performance which favour the survival and growth of the young. Similar processes no doubt underlie cold-adaptation in free-living populations, but the extent to which the changes we have recorded are general in such populations remains to be determined.

To maternal effects our present findings add an enhanced paternal performance. Maynard Smith¹² asks why male mammals that look after their young do not lactate. Perhaps the versatile house mouse, if bred for long enough in a cold climate, could achieve even this.

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Synaptic connectivity of a local circuit neurone in lateral geniculate nucleus of the cat

James E. Hamos, Susan C. Van Horn,
Denis Raczkowski, Daniel J. Uhrlich
& S. Murray Sherman

Department of Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, New York 11794, USA

Although receptive fields of relay cells in the lateral geniculate nucleus of the cat nearly match those of their retinal afferents^{1,2}, only 10–20% of the synapses on these cells derive from the retina and are excitatory^{3,4}. Many more (30–40%) are inhibitory and largely control the gating of retinogeniculate transmission^{3–7}. These inhibitory synapses derive chiefly from two cell types: intrinsic local circuit neurones and cells in the adjacent perigeniculate nucleus^{5–7}. It has been difficult to study the functional organization of these inhibitory pathways; most efforts have relied on indirect approaches^{6–12}. Here we describe the use of direct techniques to study a local circuit neurone by iontophoresing horseradish peroxidase (HRP) into it, which completely labels the soma and processes of cells for subsequent light- and electron microscopic analysis. Although the response properties of the labelled cell are virtually indistinguishable from those of many relay cells⁶, its morphology is typical of 'class 3' neurones¹³ (see Fig. 1 legend), which are widely believed to be interneurons^{9–11} (but see ref. 12). Here, we refer to the cell as a 'local circuit neurone', which allows for the possibility of a projection axon, rather than as an 'interneuron', a term that commonly excludes a projection axon. We find that the labelled cell has a myelinated axon, but that the axon loses its myelin within 50 μ m of the soma and has not yet been traced further. The dendrites of the labelled cell possess presynaptic terminals that act as intrinsic sources of inhibition on geniculate relay cells. We also characterize other morphological aspects of this inhibitory circuitry.

Our methods have been described previously^{4,12}. Briefly, a cat was anaesthetized, paralysed and prepared for neurophysiological recording with stimulating electrodes positioned across the optic chiasm. We used a bevelled micropipette, filled with HRP and KCl, to record the response properties of a geniculate cell in lamina A, impale the cell and iontophorese HRP into it. Two neuronal classes, X and Y cells, reside in laminae A and A₁^{1,2,12}. The labelled neurone responded on all physiological tests like a typical X relay cell (see Fig. 1 legend). Several hours

after HRP iontophoresis into the cell, the cat was deeply anaesthetized and perfused with fixative. We coronally sectioned the lateral geniculate nucleus at 50 μ m and reacted the sections with diaminobenzidine and 1% CoCl. The four sections containing the labelled neurone were osmicated, dehydrated and embedded in plastic resin. We fully reconstructed the neurone from the light microscope (Fig. 1) and then obtained a continuous series of thin sections for electron microscopy from the two 50- μ m-thick sections containing the labelled soma plus most of its dendritic arborization.

To characterize synaptic terminals in the lateral geniculate nucleus, we adopted Guillery's criteria and nomenclature³. These terminal types include: RLP (round vesicles, large profile and pale mitochondria), which derive from retina, form asymmetrical contacts and are excitatory; RSD (round vesicles, small profile and dark mitochondria), which mostly derive from cortex, form asymmetrical contacts and are excitatory; and F (flattened or pleomorphic vesicles), which are thought to derive from local circuit neurones and/or neurones of the perigeniculate nucleus, form symmetrical contacts and are inhibitory. F terminals can be subdivided into F1, which have a darker matrix, are never postsynaptic to other terminals and possess consistently flattened and densely packed vesicles; and F2, which have a lighter matrix, are both pre- and postsynaptic to other terminals and possess more pleomorphic and sparsely distributed vesicles. Finally, we recognized the relatively rare RLD (round vesicles, large profile and dark mitochondria) terminals¹⁴, which form asymmetrical contacts, are probably excitatory, and may derive from intrinsic collaterals of relay cell axons (refs 4, 12, 14 and our unpublished observations).

The labelled dendritic terminals contain sparsely distributed synaptic vesicles and form symmetrical synapses clearly distinguishable from the asymmetrical synapses made by nearby RLP or RSD terminals (Figs 2c, 3a). Thus, although the vesicle morphology is often indistinct because of the HRP (Figs 2c, 3a–e), the labelled terminals are clearly F terminals. Evidence presented here indicates that they are F2 terminals. Output from the dendritic terminals focuses on dendritic appendages of other geniculate neurones (Figs 2c, 3a). Figure 4 illustrates the innervation pattern from a labelled terminal cluster to the eight dendritic appendages of one postsynaptic cell. Labelled terminals provide nine synapses to varicosities from five of these appendages and contact no other adjacent structure. In this region, the postsynaptic cell receives 40 synapses from unlabelled F terminals, 23 of which clearly derive from F2 terminals (Fig. 2c). Synapses from several local circuit neurones can thus converge onto single cells. Inputs to labelled terminals originate primarily from RLP, F and occasional RSD terminals (Figs 3, 4a). Some also originate from RLD terminals (Fig. 3e), which

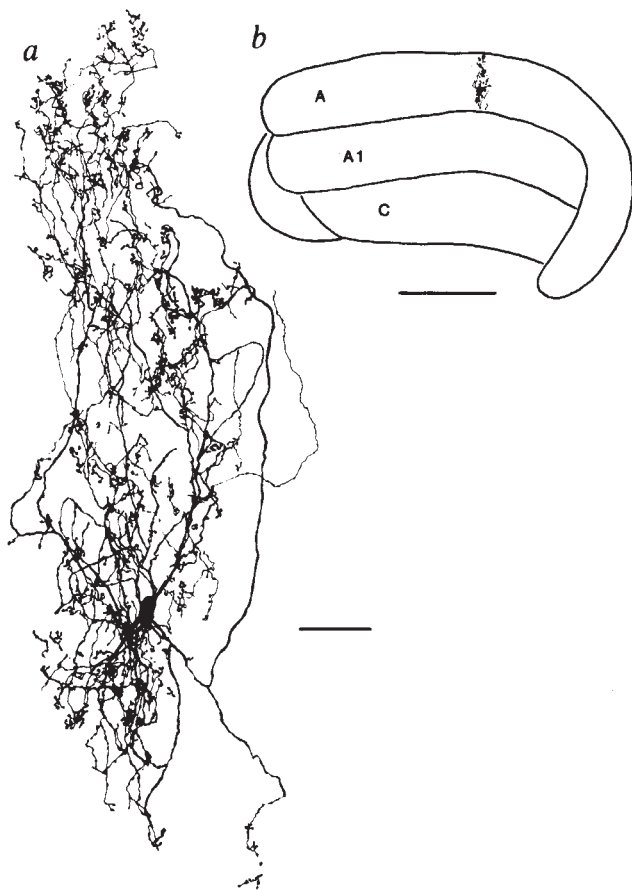


Fig. 1 Light microscopic reconstruction of a HRP-labelled neurone. The cell exhibited response properties typical of X relay cells, including linear summation in response to visual stimuli, a small on-centre receptive field (0.7° centre diameter at an eccentricity of 8° from the area centralis), and a monosynaptic response latency of 1.9 ms to stimulation of the optic chiasm^{1,2}. *a*, High-power drawing of neurone showing class 3 morphological features¹⁰. These include several thin, sinuous dendrites that radiate from a small soma ($180\ \mu\text{m}^2$ in cross-sectional area) and fine processes that issue from the dendrites to arborize into multi-lobed swellings. These swellings have presynaptic specializations and are thus dendritic terminals. Scale bar, $50\ \mu\text{m}$. *b*, Low-power drawing, showing the laminar location of the injected local circuit neurone in a coronal section through the lateral geniculate nucleus. The dendritic arborization of the cell, which is oriented perpendicular to the laminar boundaries, spans the entire depth ($550\ \mu\text{m}$) of lamina A but extends only $150\ \mu\text{m}$ in the mediolateral and rostrocaudal axes. Scale bar, $1.0\ \text{mm}$.

contact three different clusters (among the 12 analysed) of labelled dendritic terminals. This is a remarkable density of RLD terminals given their general scarcity in laminae A and A1 (unpublished observations).

Many labelled dendritic terminals enter into complex synaptic circuits, such as synaptic triads, in which a labelled terminal is postsynaptic to an RLP terminal and both terminals contact the same postsynaptic appendage (Fig. 3*a*). An RLD terminal occasionally replaces the RLP terminal in these triads. Some labelled dendritic terminals participate in serial synapses, in which the labelled terminal is both postsynaptic to an unlabelled F1 terminal and presynaptic to a dendritic appendage.

We identified the labelled dendritic terminals as F2 terminals for several reasons. These include their sparse distribution of vesicles, their symmetrical contacts and their synaptic relationships, particularly their triadic relationships^{3,4,8,15}. This is the first definitive evidence that F2 terminals are of dendritic origin, a widely hypothesized conclusion⁸ that has not been unambiguously demonstrated until now (see, for example, ref. 15).

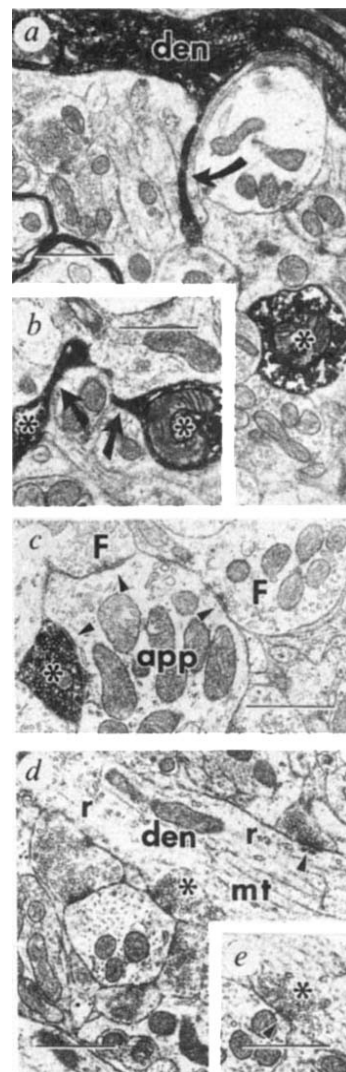


Fig. 2 Comparison of dendritic terminals from the labelled local circuit neurone with a presynaptic dendrite from an unlabelled neurone. *a*, Electron micrograph of a fine process (arrow) emanating from a secondary dendrite (den) of the local circuit neurone. In nearby sections, this process arborizes into dendritic terminals (asterisk; see also *b*, *c*). *b*, Section next to *a*, indicating branches (arrows) emanating from the process in *a* and leading to vesicle-filled terminals (asterisks). *c*, Section near *a* and *b*. The labelled terminal (asterisk) is vesicle-filled and forms a synaptic contact (arrowhead) onto a dendritic appendage (app) of an unlabelled geniculate neurone. The symmetrical contact formed by the labelled terminal is indistinguishable from those of other unlabelled F2 terminals (F) formed onto the same postsynaptic appendage (arrowheads). *d*, Electron micrograph of an unlabelled process (that is, not from the labelled neurone) with features of a presynaptic dendrite¹⁶. Note the cluster of synaptic vesicles (asterisk; see also *e*), ribosomes (r), microtubules (mt) and the site of a synaptic input (arrowhead) to the dendrite (den; other synaptic inputs are evident in adjacent sections). *e*, Section next to *d*, indicating symmetrical synapse from the presynaptic dendrite (arrowhead) to another unlabelled, medium-sized dendrite. Such dendro-dendritic contacts have been described previously for the cat lateral geniculate nucleus¹⁶ and they clearly differ from those formed by the labelled neurone reported here. Scale bars, $1.0\ \mu\text{m}$.

Furthermore, the relatively common F2 terminals are morphologically distinct from another type of quite rare dendro-dendritic synapse previously documented for the cat's lateral geniculate nucleus¹⁶. For these other dendro-dendritic contacts, the presynaptic element is a vesicle-filled dendritic shaft that contacts other dendritic shafts in simple synaptic arrangements (Fig. 2*d*, *e*).

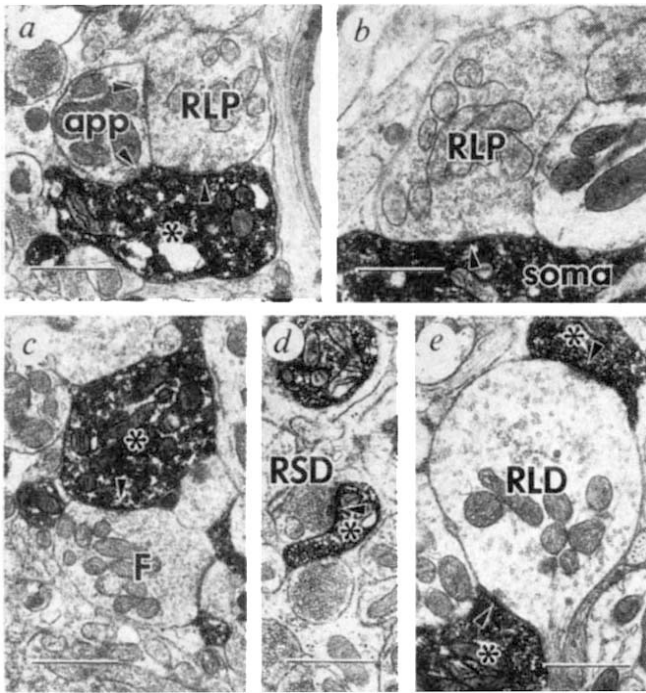


Fig. 3 Electron micrographs of synapses involving the local circuit neurone. *a*, Triadic synaptic arrangement involving a retinal terminal (RLP), an unlabelled dendritic appendage (app), and a labelled dendritic terminal (asterisk). The retinal terminal forms synapses on both the labelled dendritic terminal and an adjacent unlabelled dendritic appendage (arrowheads). This is the most common form of retinal input to the local circuit neurone. The labelled terminal contacts the appendage with a symmetrical synapse (arrowhead) and is thus equivalent to the F2 component of a synaptic triad (see text). *b*, Retinal terminal (RLP) forming a synapse (arrowhead) directly onto the soma of the local circuit neurone. This synapse plus three retinal inputs to spine-like protrusions from the soma (not illustrated) represent the only examples to date of retinal inputs contacting the local circuit neurone in a region other than a dendritic terminal. *c-e*, Other examples of synaptic inputs (arrowheads) to labelled dendritic terminals. Each is less common than retinal input (for example, in *a*) and are shown in descending relative frequency, from F terminals (*c*; most commonly from the F1 variety), RSD terminals (*d*) and RLD terminals (*e*). Scale bars, 1.0 μm .

The postsynaptic targets of the labelled dendritic terminals (for example, Fig. 4c) are almost certainly X relay cells rather than Y relay cells or other local circuit neurones. X relay cells have many dendritic appendages that receive many inhibitory (F) and retinal (RLP) inputs, whereas Y relay cells do not exhibit such features^{4,12}. Also, synaptic triads and innervation from F2 terminals are common to X relay cells and rare for Y relay cells⁴. Finally, the dendritic morphology and pattern of synaptic inputs of the postsynaptic cells differ markedly from such features of the labelled cell.

The labelled dendritic terminals are connected to one another and to dendritic shafts by long, slender processes (Figs 2a, b; 4). Processes from the dendritic shafts are 0.08–0.25 μm in diameter and extend for 1.0–7.4 μm before arborizing into the terminal clusters. Processes connecting the terminals are 0.03–0.33 μm in diameter and extend up to 4 μm . Moreover, portions of the dendritic shafts between clustered terminals are also quite long and slender. Because of the expected high impedance of such connecting processes, the synaptic circuits of dendritic terminals may be electrically isolated from one another and from the dendritic shaft. As postsynaptic potentials derive from conductance changes in the postsynaptic membrane, the high impedance of the terminal and its connecting processes would severely restrict the amount of synaptic current flowing through

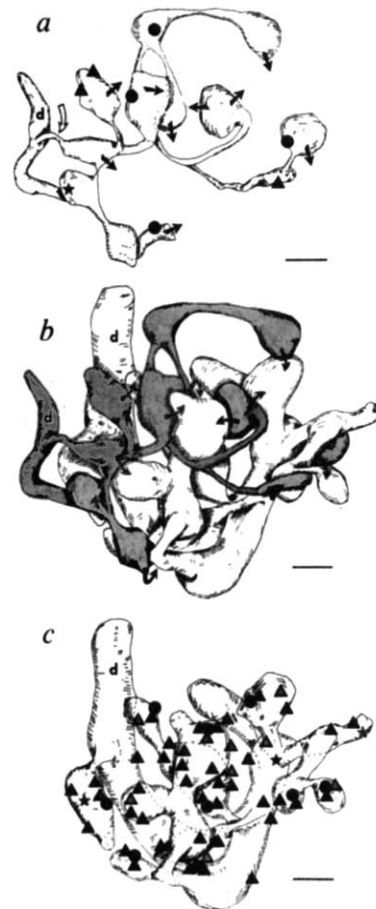


Fig. 4 Three-dimensional reconstructions from serial electron micrographs of a labelled cluster of dendritic terminals, including the stem dendrite and the single unlabelled group of dendritic appendages postsynaptic to these terminals. *a*, Labelled processes from the local circuit neurone. A small dendrite (*d*) from the labelled neurone emits a fine process (open arrow) that arborizes into 12 swellings, which are the dendritic terminals. The terminals receive inputs from RLP terminals (circles), unlabelled F terminals (triangles) and an RSD terminal (star); the labelled terminals also form nine synaptic outputs (closed arrows). *b*, Combined reconstruction of the labelled presynaptic processes (from *a*; stippled) and unlabelled postsynaptic dendritic appendages (from *c*; open). The synapses from the terminals (closed arrows as in *a*) are formed onto five appendages from a single unlabelled neurone presumed to be an X relay cell (see text). *c*, Unlabelled postsynaptic dendrite (*d*) with its eight appendages. These dendritic structures receive nine synapses from RLP terminals (circles), nine from labelled F terminals (stippled triangles; these correspond to arrows in *a* and *b*), 40 from unlabelled F terminals (filled triangles) and three from RSD terminals (stars). Triadic synapses ($n=16$; see text) are indicated by overlapping pairs of symbols for RLP and F synapses. Scale bars, 1.0 μm .

the terminals¹¹. The limitation of current flow and the attenuating properties of the connecting processes would dramatically limit the amplitude of the postsynaptic potentials conducted to the dendritic shaft and soma. Calculations based on our morphometric measurements and a passive electrical model of the postsynaptic membranes suggest that activation of an excitatory synapse located on a dendritic terminal would produce a postsynaptic potential seen at the dendritic shaft and soma that would be 10–100 times smaller than one produced from activity of an equivalent synapse located on a nearby dendritic shaft¹⁷. Such electrical isolation implies that the clusters of dendritic terminals operate in very local microcircuits. It is thus interesting that the cell exhibits action potentials. An action potential could passively depolarize the entire dendritic arbor, including the

dendritic terminals, thereby changing the gain of transmission through these terminals in response to their synaptic inputs. Hence, the firing rate of the parent cell could control the state of these microcircuits.

However, electrical isolation of the dendritic terminal clusters is difficult to reconcile with certain properties of the labelled cell. We reconstructed the pattern of retinal inputs onto two-thirds of the labelled soma and on two of its three primary dendrites plus their daughter branches up to 60 μm from the soma. This represents the most dense region of retinal synapses found on relay cells⁴. All but four of the retinal synapses contact the labelled dendritic terminals as described above. The exceptions include one retinal synapse formed directly onto the soma (Fig. 3b) and three retinal synapses on short spine-like extensions of the soma (data not shown). Such somatic sites of retinal synapses are extremely rare in geniculate neurones (refs 3, 4; our unpublished observations). This pattern of retinal inputs contrasts sharply with that seen on X relay cells, for which hundreds of retinal synapses are formed proximally on dendritic shafts and short appendages⁴. Despite the presumed electrical isolation of the vast majority of retinal inputs to the labelled cell, it responded vigorously to visual stimulation and optic chiasm shock, much like an X relay cell.

That the labelled cell and identified X relay cells exhibit such striking morphological differences despite similar response properties seems particularly interesting and deserves further investigation. We suggest at least three explanations: (1) Many retinal synapses may be located on unreconstructed regions of the labelled neurone. (2) The few somatic inputs, particularly those on spine-like processes, may activate the cell effectively. Miller *et al.*¹⁸ recently suggested that active processes in spines may significantly amplify postsynaptic potentials, so that a few retinal synapses strategically located on somatic spines might effectively discharge the postsynaptic neurone. (3) The dendritic terminals may not be isolated from each other, because our assumption that dendrites and their processes have passive electrical properties is invalid.

Here, we have described some aspects of intrinsic circuitry for the X pathway. The response properties of the labelled local circuit neurone indicate that it is innervated and vigorously driven by retinal X axons. The labelled dendritic terminals are F2 terminals and the profiles postsynaptic to them seem to derive from X relay cells. In the synaptic triads, the local circuit neurone and X relay cells are innervated by some of the same retinal X axons. This is a plausible morphological substrate of feed-forward inhibition, for which there is physiological evidence⁵⁻⁷. Because of other synaptic inputs found on clusters of labelled dendritic terminals, we further conclude that the feed-forward circuit is partially under the control of cortical (RSD), other inhibitory (F) and other relay cell (RLD) inputs. Important features that remain to be determined include a comparison of the dendritic and axonal synaptic outputs of the cell, the extent to which clusters of dendritic terminals from the same local circuit neurone are electrically isolated and the effect of action potentials in the parent cell on transmission in the dendritic terminals. Similar circuitry for the Y pathway has not yet been described, and it may be qualitatively different. Inhibitory inputs to Y relay cells are predominantly associated with F1 terminals⁴, which may derive from extrinsic sources, such as the perigeniculate nucleus¹⁹.

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Presynaptic control of synaptic channel kinetics in sympathetic neurones

Lawrence M. Marshall

Department of Physiology, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514, USA

The kinetic properties of synaptically activated ion channels are an important determinant of the duration of the synaptic currents that produce postsynaptic potentials in autonomic neurones¹⁻³ and skeletal muscle⁴⁻⁶. In the two types of principal neurones in frog sympathetic ganglia, B and C cells, a twofold difference in the mean open time of the nicotinic acetylcholine (ACh-gated) ion channels accounts for the twofold difference in the decay rate of their fast excitatory postsynaptic currents (e.p.s.cs)³. The B and C cells are selectively innervated by two distinct classes of cholinergic preganglionic axons called B and C fibres, respectively⁷. The present study examined the influence of the preganglionic nerve on the expression of synaptic ion channel properties in sympathetic neurones. B cells were denervated surgically and allowed to become innervated solely by preganglionic C fibres. These B cells, innervated by C fibres, acquired slowly decaying e.p.s.cs and long channel open times, characteristics normally seen in C cells only. These findings provide the first evidence that the kinetic properties of postsynaptic channels can be determined by the particular class of axon innervating a neurone.

Adult bullfrogs (*Rana catesbeiana*) were anaesthetized by immersion in a 0.1% solution of tricaine for 1 h. A 1-2-mm section of the interganglionic trunk between the 6th and 7th paravertebral ganglia was removed bilaterally. This surgery interrupted only the preganglionic B axons because the preganglionic C axons enter the sympathetic chain at the more distal 7th and 8th spinal segments^{7,8}. For electrophysiological examination, the 7th-10th sympathetic ganglia and associated spinal nerves were removed and pinned in a recording chamber filled with Ringer's solution³. Suction electrodes were applied to the 7th and 8th spinal nerves to stimulate preganglionic C fibres, to the sympathetic chain above the 7th ganglion to stimulate the preganglionic B fibres, and to the sciatic nerve for antidromic stimulation of ganglion cell axons. Nerve conduction velocity was estimated by the ratio of the nerve length to the response latency (time between stimulus and response). To ensure accurate identification of neurones, experiments were limited to cells possessing antidromic conduction velocities exceeding 1 m s⁻¹ for B cells and less than 0.25 m s⁻¹ for C cells. This avoided the possibility of confusing some more slowly conducting B cells with C cells⁹.

Neurones in the 9th and 10th ganglia of operated and normal frogs were impaled with two microelectrodes under visual control, and voltage clamped³ at -50 mV. In the traces shown in Fig. 1, an antidromic shock for cell identification is followed 70 ms later by an orthodromic stimulus. Figure 1a shows representative anti- and orthodromic responses recorded from a normal B cell; a short-latency antidromic response is followed by a short-latency orthodromic e.p.s.c. evoked by stimulating the fast-conducting preganglionic B fibres. A typical recording from a C cell (Fig. 1b) is easily distinguished from that of a B

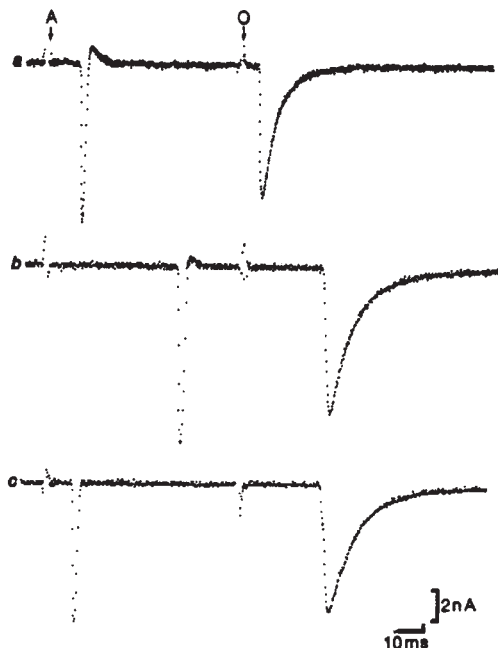


Fig. 1 Examples of antidromic and orthodromic responses of a normal B cell (*a*), a normal C cell (*b*) and a B cell innervated by a preganglionic C axon (*c*). The first response in each digitized trace is the antidromic impulse current evoked by stimulation at A to identify the ganglion cells; the antidromic response latency is short for B cells (*a, c*) and long for C cells (*b*). The second response in each trace is the fast e.p.s.c. evoked by stimulation of the appropriate preganglionic nerves at O. The latency of the e.p.s.c. indicates the type of innervating preganglionic axon; a short latency for B fibres (*a*) and a long latency for C fibres (*b, c*). Twenty-one days after surgery the B cell (*c*) receives C fibre input and has a slowly decaying e.p.s.c. similar to that of a normal C cell (*b*). Neurones were voltage-clamped at -50 mV, 21 – 23 °C.

cell by the longer latencies of both the ortho- and antidromic responses, and by the more slowly decaying phase of the e.p.s.c.

Three weeks after transection of preganglionic B axons, B ganglion cells were identified by antidromic conduction velocity (>1 m s $^{-1}$) and found to be innervated only by preganglionic C fibres, apparently from axon sprouting¹⁰. Figure 1*c* shows a typical response of a B cell (short antidromic latency) to orthodromic stimulation of C fibres (long orthodromic latency); the B cells now have slowly decaying e.p.s.cs which are indistinguishable from those of normal C cells³ (Fig. 3). Semilogarithmic plots in Fig. 2 show this clear change in the exponential decay time constant of the e.p.s.c. when a B cell is innervated by a preganglionic C fibre.

One explanation is that this change in the time course of the e.p.s.c. is caused by denervation and subsequent reinnervation, regardless of the type of preganglionic axon. To test this possibility, ganglia were totally denervated by cutting the B fibres as usual, and also severing the rami communicantes of the 7th and 8th ganglia which carry all the preganglionic C axons. Within 7–14 days, the B cells were reinnervated solely by B fibres, probably because the B axons are larger and grow much faster than the C axons. In this case, the B cells had a mean e.p.s.c. decay time constant that was not significantly different from that of normal B cells (Fig. 3). This finding indicates that the slower e.p.s.c. decay is a specific response to innervation by axons of preganglionic C neurones.

It is also possible that the kinetics of the e.p.s.c. in C fibre-innervated B cells are changed only transiently and will in time become normal. To maintain C fibre innervation of B cells, the operation was repeated in four frogs after 4–6 weeks to prevent the return of the preganglionic B axons¹⁰. All 45 B cells examined 90–110 days after the initial denervation had maintained the slow e.p.s.c. decay time normally seen in C neurones only.

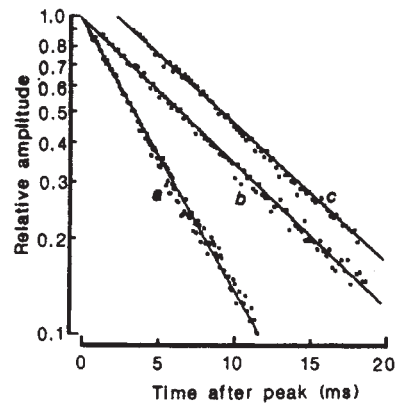


Fig. 2 Exponential decay of e.p.s.cs from a normal B cell (*a*), a normal C cell (*b*) and a B cell with synaptic input from a C fibre (*c*). The amplitudes of e.p.s.cs from the traces in Fig. 1 are plotted on a semilogarithmic scale as a function of time after the peak and fitted to a least-squares regression line (continuous line). The decay time constants, calculated as the inverse slope of the regression line, are: *a*, 5.1 ms; *b*, 9.5 ms; *c*, 9.9 ms. Plot *c* is displaced 2.5 ms along the time axis for clarity.

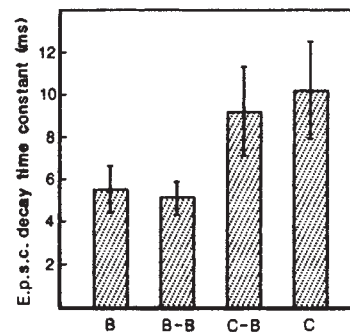


Fig. 3 Summary bar graph of mean e.p.s.c. decay time constants. When B cells are reinnervated by the native B fibres (B-B), the e.p.s.c. decay is not significantly different ($P > 0.05$) from that of B cells from normal (control) ganglia (B). Twenty-one days after the preganglionic B fibres have been severed, the B cells are innervated by C fibres (C-B) and have e.p.s.c. decay time constants that are not significantly different ($P > 0.05$) from those of normal C cells (C). Error bars represent ± 1.0 s.d. The number of cells examined are: *a*, 46; *b*, 14; *c*, 23; *d*, 28. Statistics are from Student's *t*-test.

This slower e.p.s.c. decay could be a result of the longer mean open time of the nicotinic ACh-gated channels as seen in normal C cells³. Mean channel open time was estimated by spectral analysis of membrane current fluctuations produced by a steady iontophoretic application of ACh from a micropipette positioned 30–40 μ m above the neuronal surface⁸. Activation of muscarinic ACh receptors was blocked by 0.5 μ M atropine sulphate present in the Ringer's solution¹¹. Estimates of single channel conductance were similar for normal B cells, normal C cells and C fibre-innervated B cells (Table 1). The mean channel open time of C fibre-innervated B cells was not significantly different from that of normal C cells and nearly twice as long as that of normal B cells (Table 1). The close agreement between the mean channel open time of 9.5 ± 1.8 ms ($\pm$ s.d., 8 cells) and the e.p.s.c. decay time of 9.2 ± 2.1 ($\pm$ s.d., 23 cells) suggests that the synaptic current follows the relaxation of channels opened by a very brief exposure to ACh⁵. Preliminary results (L.M.M., unpublished) show no increase in the mean channel open time when B cells are totally denervated. Thus, the change to longer channel open time seen here probably occurs after innervation by preganglionic C axons.

Table 1 Channel properties obtained from spectral analysis³ of ACh-induced membrane current fluctuations at -50 mV, 21–23 °C

	Open time (ms)	Conductance* (pS)	No. cells
	mean $\pm$ s.d.	mean $\pm$ s.d.	
Normal B cells	5.2 $\pm$ 0.9	17.3 $\pm$ 3.3	22
Normal C cells	9.8 $\pm$ 2.0	16.9 $\pm$ 2.7	15
B cells with C fibre input	9.5 $\pm$ 1.8	17.5 $\pm$ 3.1	8

* Single-channel conductance values are not significantly different ($P > 0.3$; Student's *t*-test).

Taken together, these results argue strongly that the expression of specific channel kinetics at synaptic contacts on sympathetic neurones depends on the type of innervating axon. Such a neural control of channel kinetics may be similar to the situation during the formation of the neuromuscular junction, where innervation by motor axons is accompanied by a reduction in the open time of ACh-gated channels^{12–14}. Experiments are in progress to examine the effects of electrical activity on channel kinetics and

to follow the temporal sequence of changes in channel properties that occur during reinnervation of these sympathetic neurones. Future studies of frog sympathetic ganglia may provide important insights into the mechanisms by which neurones interact to specify the functional properties of synapses.

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A crucial epileptogenic site in the deep prepiriform cortex

Salvatore Piredda & Karen Gale

Department of Pharmacology, Schools of Medicine and Dentistry, Washington, DC 20007, USA

Antagonists of γ -aminobutyric acid (GABA)- or glycine-mediated neurotransmission, muscarinic cholinergic agonists, and excitatory amino acids and their analogues are all considered to be potent chemoconvulsant agents. However, although systemic injections of these agents have been used to create experimental models of generalized epilepsy, there has been no identification of a specific locus at which any of these drugs act to initiate generalized seizures. We recently located a forebrain region from which seizures can be elicited by the GABA antagonist bicuculline¹, and now report that manipulations of excitatory amino acid transmission and cholinergic transmission can also elicit seizures from this site. Bilateral clonic seizures can be elicited after unilateral application of picomole amounts of bicuculline, kainic acid or carbachol and micromole amounts of glutamate. Local application of the GABA agonist muscimol prevents the appearance of seizures on subsequent microinjection of all convulsant agents examined, whereas local application of the muscarinic antagonist, atropine, only prevents seizures induced by carbachol. This region is therefore a site of action for the epileptogenic effects of neuroactive agents with diverse mechanisms of action; it may also represent a site at which GABA agonists could function therapeutically to control epileptogenesis.

Although certain brain regions such as hippocampus and amygdala are especially sensitive to the seizure-inducing actions of some epileptogenic agents as evidenced electrographically^{2–4} or metabolically^{2,3} experiments in which a single application of a low dose of a chemoconvulsant is placed directly into these and other brain regions have generally failed to elicit bilateral motor seizures. Intrahippocampal or intraamygdala application of relatively high doses of bicuculline or carbachol do not produce clinical seizure activity, despite the appearance of electrographic signs of seizure discharge under certain conditions^{5–7}. Intracerebral application of nanomole amounts of kainic acid can produce strong and long-lasting bilateral motor seizures^{8,9} but this effect is not anatomically selective, as it can be elicited from many injection sites in the forebrain¹⁰. Although high doses of strychnine and bicuculline have been reported to evoke contralateral focal clonus after application to motor cortex, bilateral seizures did not occur with these treatments^{11,12}. We therefore investigated the effects of the application of chemocon-

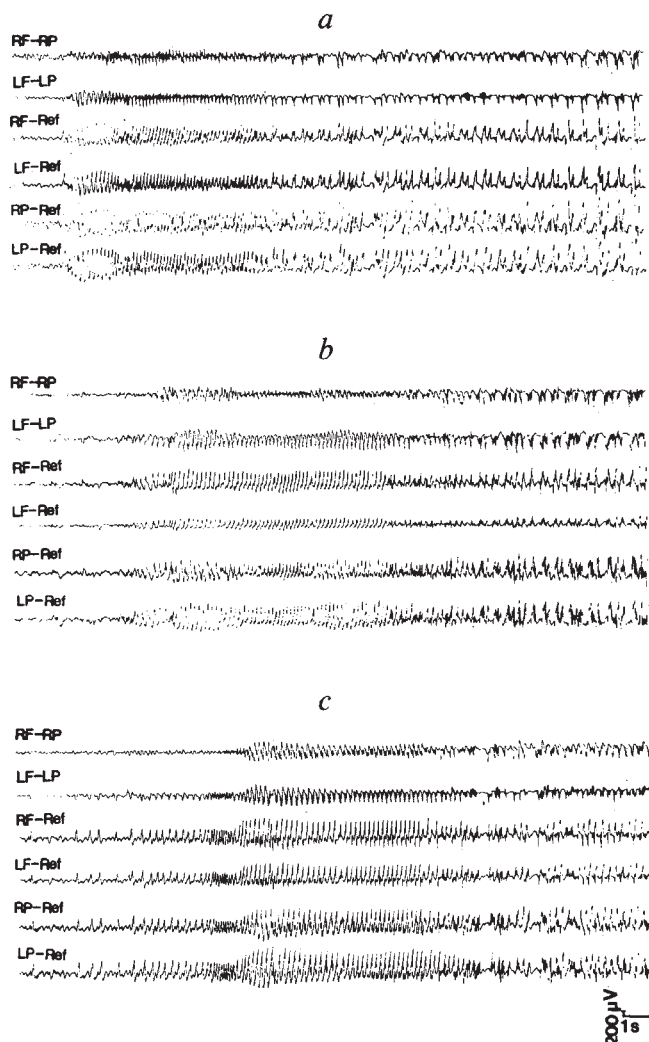


Fig. 1 Electrographic recording of generalized seizures elicited from deep prepiriform cortex by bicuculline (a), carbachol (b) and kainic acid (c). The cortical leads (Plastic Product Company, Roanoke, Virginia) were connected to screws implanted over the two frontal and the two parietal regions, and with an indifferent electrode screwed over the cerebellar region. Abbreviations: R, right; L, left; F, frontal; P, parietal; Ref, reference. The behavioural correlate of these EEG traces are rearing and clonus of both forelimbs. The seizures started 2–3 min after the end of drug injection.

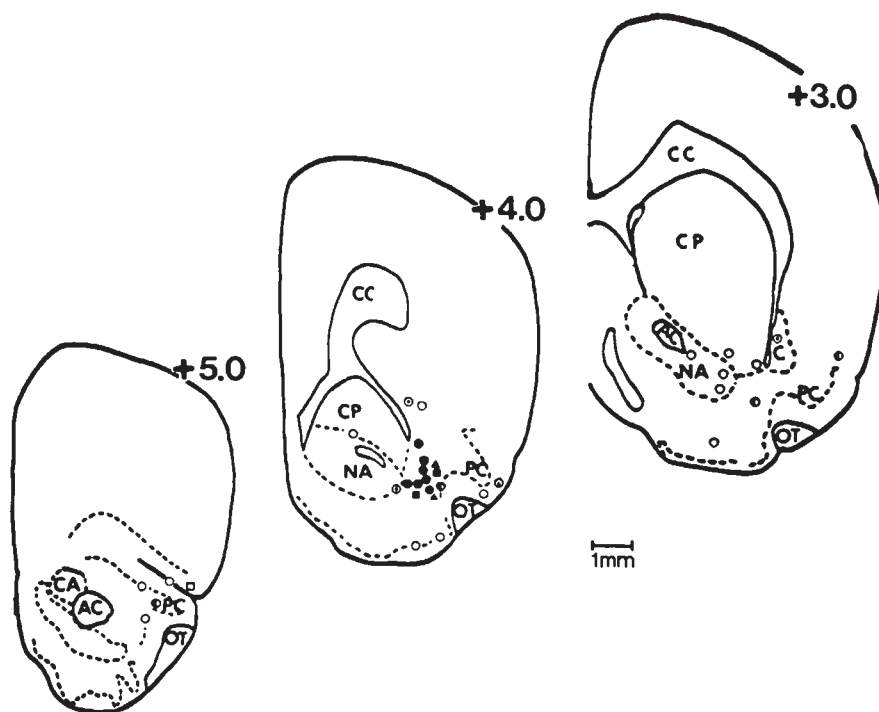


Fig. 2 Schematic diagram of coronal sections of rat brain based on the atlas of Pellegrino and Cushman¹⁸. Large numbers on the right side of the figures indicate rostrocaudal level of section in mm anterior to bregma. See text for coordinates for deep prepiriform cortex. The locations of the tips of the injection cannula tracts were determined histologically in 50- μ m coronal sections stained with neutral red. Circles indicate positions of cannula tips in rats treated respectively with bicuculline, carbachol or kainic acid. Where injection sites for kainic acid or carbachol did not overlap with sites for bicuculline, these are denoted with triangles (kainic acid) or squares (carbachol). Different symbols are used for different levels of seizure score and/or for seizure latency (measured from beginning of infusion). $\bullet$, $\blacksquare$, $\blacktriangle$, Maximal responses, score 4-5 and latency <7 min; $\odot$, partial or delayed response, score 4-5 and latency >7 min or score 2-3; $\circ$, weak or barely perceptible response, score 1; $\square$, $\square$, no response. AC, Anterior commissure; C, claustrum; CC, corpus callosum; CP, caudate-putamen; NA, nucleus accumbens; OT, lateral olfactory tract; PC, piriform cortex.

vulsant agents into forebrain regions not previously examined in this context.

Male Sprague-Dawley rats weighing 360-380 g were surgically prepared under Equithesin anaesthesia at least a day before the experiments; screw electrodes for electroencephalogram (EEG) recording were connected to the skull and 22-gauge stainless steel guide cannulae were stereotactically implanted. Bicuculline methiodide, kainic acid and carbachol were dissolved in saline at 100 ng μ l⁻¹, strychnine sulphate and sodium glutamate were dissolved in distilled water at a concentration of 150 and 147 ng μ l⁻¹, respectively. The drugs were then injected (0.125 μ l min⁻¹) via 28-gauge injection cannulae which extended at least 1 mm below the tip of the guide cannulae. The most consistently effective site for obtaining seizures with each of the drugs tested, with the exception of strychnine, corresponds to the following stereotaxic coordinates when the incisor bar is 5 mm above the interaural line: 4 mm anterior to bregma, 3.0 mm lateral to midline and 6.5 mm below dura (see Fig. 2).

All animals injected with bicuculline (49 pmol) or kainic acid (117 pmol) and 8 of 13 injected with carbachol (136 pmol) into

this region showed bilateral clonic seizures before or within 3 min after the end of the infusion (Table 1). Strychnine infusions did not induce seizures even at high doses (450 pmol). The seizures induced by the focal injections of kainic acid, bicuculline and carbachol usually began with myoclonic jerks which rapidly progressed to clonic movements of the forelimb contralateral to the injected side. Occasionally, mouth and facial clonus were observed. In most cases, seizures developed within a few minutes to a stage in which both forelimbs showed clonus, with rearing and falling occurring (stages 4 and 5). The duration of each clonic seizure ranged from 5 to 30 s. In all rats, more than one seizure was observed during the 30 min following the infusion of the drug and in some cases seizures induced by kainic acid recurred continually for 6-8 h. Although injections were always unilateral, the EEG showed bilateral single spikes or sharp waves concomitantly with each myoclonic jerk, and prolonged bilaterally synchronous spiking activity during the clonic seizures (see Fig. 1). Recordings made with depth electrodes in other rats revealed that the epileptiform activity started in the form of single spikes near the injection site; these spikes

Table 1 Anticonvulsant effect of atropine and muscimol against bilateral motor seizures elicited from deep prepiriform cortex (DPC) by bicuculline, carbachol and kainic acid

		Muscimol			Atropine	
		Control	39 pmol	79 pmol	143 pmol	20 nmol
Bicuculline 49 pmol	Incidence of clonus	11/11	0/3		3/3	3/3
	Seizure score	4.1 $\pm$ 0.1	0*		4.0 $\pm$ 0	4.7 $\pm$ 0.3
Carbachol 136 pmol	Incidence of clonus	8/13	0/6		1/10	
	Seizure score	2.7 $\pm$ 0.6	0*		0.4 $\pm$ 0.1*	
Kainic acid 117 pmol	Incidence of clonus	5/5	3/3	2/6	3/3	3/3
	Seizure score	4.0 $\pm$ 0.3	4.3 $\pm$ 0.3	1.3 $\pm$ 0.8*	4.3 $\pm$ 0.3	4.3 $\pm$ 0.3

Bicuculline (49 pmol), carbachol (136 pmol) or kainic acid (117 pmol) were infused over a period of 2 min into DPC. Atropine (143 pmol or 20 nmol) or muscimol (39 or 79 pmol) were microinjected 5 min before convulsant infusions. Rats were observed for 30 min after the injection of convulsants and the seizure score and the latency of seizure were recorded using a five-point seizure score scale: (1) myoclonic jerks of the contralateral forelimb; (2) mild forelimb clonus ($\pm$ mouth and facial movements—clonus of jaw and vibrissae and head nodding) lasting at least 5 s; (3) severe forelimb clonus lasting more than 15 s; (4) rearing in addition to severe forelimb clonus; (5) rearing and falling in addition to severe forelimb clonus. A score of zero was assigned if there was no evidence of convulsive activity. Scores 4 and 5 are defined in the same way as originally described by Racine for kindled seizures¹⁶; some investigators classify seizures of this severity as generalized motor seizures¹⁹⁻²².

Significantly different from the control group (Mann-Whitney *U*-test for non-parametric grouped data, *P* < 0.05).

diffused cortically and subcortically to all regions explored. During this period, there were usually no clinical seizure correlates. Within 2–5 min, ictal activity, associated with clonic seizures, appeared with bilaterally synchronous onset in all cortical and subcortical structures explored. Infusions of saline in the same site were without effects on motor or EEG activity.

To determine site specificity, we evaluated the effect of infusions of bicuculline, carbachol or kainic acid placed 1.0–2.0 mm from the 'active' site described above: in most cases, no seizure activity was obtained (Fig. 2). In those cases in which seizures were observed, they occurred only after a significant delay (more than 7 min) and were consistently less severe than those obtained from the active site (Fig. 2).

The GABA receptor agonist muscimol (39 pmol; 0.25 μ l; 2 min), prevented both bicuculline and carbachol-induced seizures. A higher dose of muscimol (79 pmol; 0.25 μ l; 2 min) was required to prevent kainic acid-induced seizures (Table 1). Blockade of muscarinic cholinergic receptors with atropine sulphate (143 pmol; 0.25 μ l; 2 min) prevented seizures induced by carbachol, but was ineffective against seizures induced by the other chemoconvulsants, even when atropine was injected at a dose (20 nmol; 0.5 μ l; 4 min) >100 times greater than that required to block carbachol seizures (Table 1). These data, taken together with the absence of an effect of strychnine, indicate that specific receptor mechanisms mediate seizure responses at this forebrain site, and suggest that GABA receptors exert inhibitory control over neurones in this region that may be responsive to multiple excitatory influences.

As seizures induced by blockade of GABA inhibition (by bicuculline) are not prevented by atropine, it seems that the cholinergic system in this area is not necessary for seizure initiation; at least one other source of excitatory drive must function in this capacity. The excitatory amino acids are likely candidates, so we examined the effect of glutamate injections (1 μ mol; 1 μ l; 8 min) in this area. Of 5 rats tested, 3 showed generalized clonic seizures in response to injection of 1.0 μ mol glutamate. This is consistent with the possibility that glutamate or another excitatory amino-acid transmitter, such as aspartate, could be responsible for initiating seizures from this site. More extensive pharmacological studies will determine whether activation of receptors for excitatory amino acids is actually required for the seizures.

The doses of convulsants effective for producing seizures following our focal injections are considerably lower than have been previously applied to other forebrain areas in attempts to produce seizures. Even in doses more than 20 times higher than the doses we used, neither bicuculline nor carbachol caused seizures when placed in amygdala or hippocampus^{6,13–15}; when placed in the cortex, these high doses have produced, at most, only focal seizure activity¹². Injections of kainic acid in doses 30–40 times higher than the dose we used are required to induce bilateral motor seizures from forebrain sites such as hippocampus⁹, amygdala⁸ or striatum¹⁰. It is therefore possible that seizures obtained following high doses of convulsants placed elsewhere in the forebrain may, in fact, be caused by diffusion of small amounts of drug to the region we have identified.

Some of the chemoconvulsants that we have examined have been previously shown to elicit convulsions on repeated focal injections in sites such as amygdala or neocortex. In particular, kindled seizures are obtained with repeated injections of bicuculline (nanomole amounts) but not carbachol into cortex⁶, or with repeated injections of carbachol (nanomole amounts), but not bicuculline, into amygdala^{6,14}. It is apparent that the site from which we have elicited seizures is distinct from these sites in three important ways: (1) it is considerably more sensitive (seizures are elicited with lower doses of chemoconvulsants than required to evoke seizures in the kindled animals); (2) it does not require repeated stimulation in order to elicit bilateral motor seizures; and (3) it is responsive to chemoconvulsants with a variety of mechanisms of action. On the other hand, there is marked similarity between the motor seizures induced by kindling¹⁶ and those elicited by our focal injections, raising the

possibility that development of kindled seizures might depend on a progressive facilitation of activity in a neuronal circuit involving the site that we have identified. In an analogous fashion it is possible that, following systemic injection of chemoconvulsants, the early signs of seizure activity observed electrographically in areas such as hippocampus and amygdala may be a secondary consequence of activation of neurones in the vicinity of our focal injection site.

The fact that bilaterally synchronous seizure activity consistently and rapidly occurred following unilateral application of the chemoconvulsants into the deep prepiriform cortex suggests that this area may be a source of excitatory drive to limbic and cortical structures of both hemispheres. Anatomical studies have demonstrated significant commissural projections from this area, as well as homolateral projections to entorhinal cortex¹⁷. Clearly, it will now be important to characterize the functional interconnections between the site that we have identified and other limbic areas thought to play a significant role in chemoconvulsant seizures. As this site in the deep prepiriform cortex seems able to trigger bilateral motor seizures in response to manipulations of cholinergic, GABAergic and excitatory amino acid-receptors, it could represent a common denominator for seizure models previously thought to be generated via distinct neural circuits.

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Inverse relationship of the durations of adjacent open and shut intervals for Cl and K channels

O. B. McManus, A. L. Blatz & K. L. Magleby

Department of Physiology, University of Miami School of Medicine, Miami, Florida 33101, USA

Ion channels in cell membranes, whether voltage-dependent or activated by ligands, make repeated transitions among open and shut states during activity^{1,2}. Information about the number of states and the transitional pathways between them can be obtained from the durations of open and shut intervals, as transitions to states of different lifetimes result in intervals of different mean durations³. If there is only one open conformation, or state, then the durations of open intervals would be independent of the durations of adjacent shut intervals. On the other hand, if a channel has two or more open states with different mean lifetimes, and if each open state is entered directly from a different shut state with a different mean lifetime, then the open intervals should be related to the adjacent shut intervals. We now report that the durations

of adjacent open and shut intervals for both a Cl channel and a large conductance Ca-activated K channel in skeletal muscle are inversely related; shorter open intervals are adjacent to longer shut intervals. These findings indicate that two or more shut states make direct transitions to two or more open states, and suggest that the lifetimes of adjacent open and shut states are inversely related.

Figure 1a shows single-channel currents⁴ through a large conductance Ca-activated K channel (BK channel)⁵⁻⁷ and a Cl channel with fast kinetics active at resting membrane potentials⁸. Closing and opening of the channels is clearly indicated by the abrupt steps in current level. Both channels display bursts of openings and have widely varying durations of open and shut intervals, suggesting multiple open and shut states.

Figure 1b presents the relationship between adjacent open and shut intervals for the BK channel. The mean duration of all open intervals adjacent to shut intervals within a specified range of durations is plotted against the mean duration of the specified shut intervals. As the specified shut intervals became longer, the mean durations of the adjacent open intervals became shorter. Similar results were obtained for the Cl channel (Fig. 1c). Because the lifetimes of the different states of a channel are exponentially distributed, it is impossible to determine which state produces any given interval. However, intervals from states with shorter lifetimes would be, on average, shorter than intervals from states with longer lifetimes. Thus, the results in Fig. 1 suggest that open states with shorter lifetimes are adjacent to shut states with longer lifetimes.

A similar inverse relationship between the durations of adjacent open and shut intervals was found when the mean shut intervals adjacent to specified ranges of open intervals were determined (Fig. 2). Results similar to those shown in Figs 1 and 2 were obtained in six out of seven membrane patches containing either a single BK or Cl channel. In the one experiment with atypical results (Cl channel), the durations of adjacent open and shut intervals appeared relatively independent, rather than inversely related.

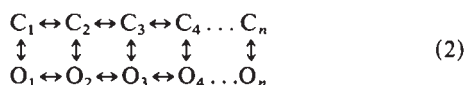
Because of the necessarily limited time resolution of single-channel data, brief intervals are not detected, leading to an increase in the observed duration of open and shut intervals^{4,9}. We have also analysed data that were corrected for missed brief intervals (manuscripts in preparation) and found an inverse relationship between the lifetimes of adjacent open and shut states for the models examined, although the magnitude of the effect was less.

The findings in Figs 1 and 2 exclude models for the BK and Cl channels in which there is only one transition pathway between the open and shut states:



where C represents closed states and O open states. Models with only one transition pathway between open and shut states require that the mean durations of adjacent open and shut intervals be independent.

The findings in Figs 1 and 2 are consistent with models in which two or more shut states make direct transitions to two or more open states. A general case for such a model is presented in scheme 2



where C_1 is the shut state of longest lifetime and O_1 is the open state of shortest lifetime. A decrease in the lifetimes of shut states from C_1 to C_n and an increase in the lifetimes of open states from O_1 to O_n would give rise to an inverse relationship between durations of adjacent open or shut intervals (Figs 1, 2). The number of states would depend on the channel. Some states may be too brief to detect or may be entered infrequently, such as O_1 and O_2 , and some of the transition rates between the states may be insignificant.

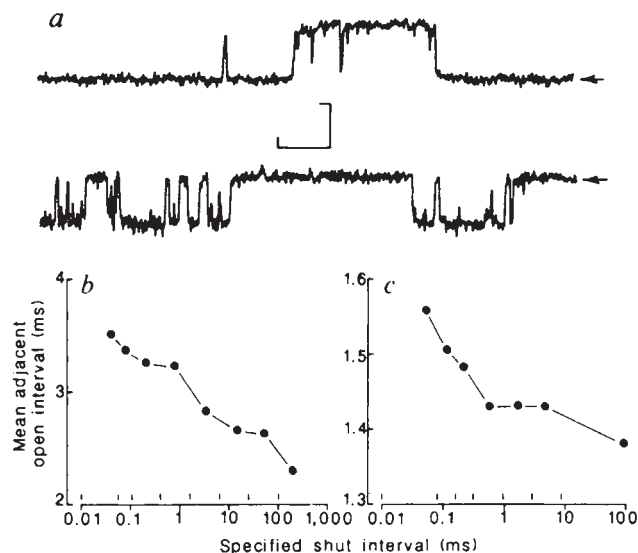


Fig. 1 Single-channel data used to determine the relationship between adjacent open and shut intervals.

a, Records of currents through a single large conductance Ca-activated K channel (BK channel, upper trace) and a single Cl channel (lower trace). Arrows indicate close-channel current level. Vertical and horizontal bars are 9 pA and 3.8 ms, respectively, for the BK channel and 5 pA and 10 ms for the Cl channel. b, c, Relationship between the durations of specified shut intervals and adjacent open intervals for BK channel (b) and Cl channel (c). The range of specified shut intervals for each plotted point is indicated by the vertical lines along the logarithmic abscissa. We analysed 64,000 intervals for the BK channel and 85,000 for the Cl channel. The inverse relationship between adjacent open and shut intervals shown here and in Fig. 2 is not caused by false events resulting from baseline noise because the filtering was sufficient to reduce baseline noise below the threshold level for detection of channel opening and closing.

Methods. a, The patch-clamp technique⁴ was used to record currents through single channels in inside-out patches of membrane excised from primary cultures of rat muscle cells¹⁷. For the BK channel the solution at the intracellular side of the membrane contained, in mM: 145 KCl, 2 TES buffer, 1 EGTA and 0.9 Ca^{2+} (free Ca^{2+} = 0.7 μM), pH 7.2. The extracellular solution was similar but contained no added Ca^{2+} . The membrane potential was held at +30 mV, intracellular side positive. Low-pass active filtering at 5 kHz (-3 dB), 24 dB per octave. Temperature, 22 °C. For the Cl channel the intracellular solution contained, in mM: 1,000 KCl, 5 TES, 0.5 EGTA, pH 7.2. The extracellular solution contained, in mM: 140 KCl, 5 TES, 0.5 EGTA and 57 μM added Ca^{2+} (free Ca^{2+} 10^{-8} M). Membrane potential, -40 mV; 3.1-kHz (-3 dB) low-pass filtering; temperature, 7.6 °C. b, c, Durations of open and shut intervals from membrane patches containing a single channel were measured by computer (PDP 11/23) and stored in sequential order. Channel opening and closing was taken to occur at the time the current crossed a threshold, typically set midway between the open and shut current levels. The stored sequential data were searched for shut intervals whose durations fell in a series of specified ranges and the mean duration of the open intervals that occurred immediately before and after the shut intervals in each range was calculated. Mean open intervals were then plotted against the mean of the shut intervals in each specified range. Correcting brief intervals for underestimation of duration introduced by the threshold-crossing measurement technique⁹ had negligible effects on the results: the corrected data were essentially unchanged from the uncorrected plotted data except for a slight shift of about one symbol diameter to the right for the first plotted symbol. The correction was even less for Fig. 2.

Scheme 2 is consistent with models for the BK and acetylcholine receptor channels that have been derived in different manners from dose-response curves, distributions of open and shut intervals and correlation of open or shut intervals¹⁰⁻¹⁵. In these models the binding of acetylcholine to the acetylcholine receptor channel or of Ca to the BK channel drives the reaction scheme to the right and down towards the longest open state. Scheme 2 also has some features in common with allosteric models that

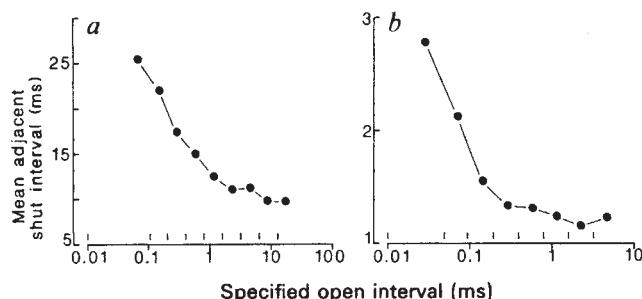


Fig. 2 Relationship between the durations of specified open intervals and adjacent shut intervals in: *a*, the BK channel and *b*, the CI channel. Experiments are the same as in Fig. 1, but the data were analysed to find the mean of the shut intervals adjacent to open intervals of specified duration. For the CI channel, all shut intervals >50 ms (~100 out of 42,500 intervals) were set equal to 50 ms to reduce excessive variation in the durations of the mean shut intervals that would result from the few long shut intervals. The same general relationship, but with greater mean shut interval duration and more variation between adjacent points was observed when this condition was not set.

have been proposed for the binding of oxygen by haemoglobin¹⁶. Thus, scheme 2 may represent a general model for at least three types of channels as well as some other types of allosteric proteins.

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Selective activation of $\text{Lyt } 2^+$ precursor T cells by ligation of the antigen receptor

I. N. Crispe, M. J. Bevan & U. D. Staerz

Department of Immunology, Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, California 92037, USA

Resting T lymphocytes may be activated either physiologically, by the specific recognition of antigen in association with molecules encoded by the major histocompatibility complex (MHC), or non-physiologically using mitogens such as concanavalin A (Con A). The former activation process is difficult to analyse because resting precursor T cells specific for a particular antigen-MHC combination can only be isolated in the presence of a large excess of bystander cells of irrelevant specificity; clonal populations of

uniform specificity are not useful for studying the activation of naive T cells because there is no reason to believe that such cloned cells ever return to the state of resting precursors. Mitogens may activate a large fraction of resting T cells, but analysis is again complicated because the target molecule(s) of most mitogens is unknown and the relationship of this kind of activation to physiological induction by antigen plus MHC molecules remains unclear. By using a monoclonal antibody specific for the antigen receptors on ~25% of all T cells of both $\text{Lyt } 2^+$ and $\text{Lyt } 2^-$ subsets, we have studied the induction of lymphokine responsiveness in resting normal T cells. This antibody, immobilized on Sepharose beads, is sufficient to activate $\text{Lyt } 2^+$ T cells, but not $\text{Lyt } 2^-$ T cells, to clonal expansion in the presence of a mixture of lymphokines (10% rat spleen Con A supernatant). We report here that clonal growth of the T cells obeys single-hit kinetics in limiting-dilution microcultures, suggesting that a single cell type is limiting. We conclude that cytotoxic T-lymphocyte (T_c) precursors require only ligation of the antigen receptor before they become responsive to lymphokines, whereas helper T-lymphocyte (T_h) precursors require additional signals.

The monoclonal antibody F23.1 recognizes the T-cell antigen receptor on ~25% of T cells in most mouse strains¹. Intact F23.1 antibody in the presence of accessory cells, and either intact antibody or F(ab')_2 fragments coupled to Sepharose beads, are mitogenic in bulk cultures for resting T cells in the presence of rat spleen Con A supernatant (ConASN). Furthermore, the majority of viable cells in such cultures are $\text{Lyt } 2^+$ T cells (U.D.S. and M.J.B., manuscript in preparation). To study the distribution of the F23.1 determinant on T cells of both $\text{Lyt } 2^+$ and $\text{Lyt } 2^-$ subsets, BALB.B (C.B) lymph-node T cells were stained with the monoclonal antibodies GK1.5 (anti-L3T4), 53.6.72 (anti-Lyt 2) and F23.1, then with a second layer of fluorescein-coupled goat anti-mouse immunoglobulin. By fluorescence-activated cell sorting (FACS) analysis, unseparated T cells were 70.0% L3T4⁺, 18.0% $\text{Lyt } 2^+$, and 23.0% carried the F23.1 determinant (Fig. 1*a, b*). Cells pre-killed with the anti-Lyt 2 antibody plus complement were 95.5% L3T4⁺, no cells were scored as $\text{Lyt } 2^+$ on restaining, and 20.9% were F23.1⁺ (Fig. 1*c, d*). Thus, both L3T4⁺ T_h precursors and $\text{Lyt } 2^+$ T_c precursors express the F23.1 determinant. This is not surprising as available evidence suggests that the two subsets of T cells use the same structural units in their antigen receptor; both express the allotypic determinant defined by monoclonal antibody KJ16-133 (ref. 2), and both rearrange and transcribe the same α - and β -chain genes³.

The induction of lymphokine responsiveness in T cells by the F23.1 anti-receptor antibody immobilized on Sepharose beads, was examined by culturing limiting numbers of C.B lymph-node T cells in the presence of approximately 1,000 such beads per half-area microwell. Bovine γ -globulin (BGG)-conjugated beads were used as a negative control. ConASN at a saturating final concentration of 10%, with residual Con A neutralized by addition of α -methyl mannoside, was used to provide lymphokines including interleukin-2 (IL-2) (ConASN at 5% was suboptimal, but higher concentrations up to 40% produced no greater effect). After 7 days of incubation, cultures were scored for clonal expansion by either measurement of ³H-thymidine (³H-TdR) incorporation or direct inspection. Similar numbers of positive cultures were detected by the two techniques. Positive cultures contained one or several clusters of typically 50-1,000 activated cells that were adhering to the F23.1-Sepharose beads (Fig. 2). Cultures with BGG-Sepharose beads never contained any such clusters. The frequencies of responding precursor cells were calculated using the Poisson distribution⁴.

Among unselected lymph-node T cells, 1 in 55 generated a clone under these conditions (range 1/55 to 1/96; 6 experiments). However, when $\text{Lyt } 2^+$ T cells were eliminated by prior treatment of T cells with the anti-Lyt 2 monoclonal antibody AD.415 plus rabbit complement, clonal precursors were either drastically reduced in frequency (other experiments) or abolished completely (Fig. 3*a*). To verify that the preparation procedure did not severely compromise the ability of $\text{Lyt } 2^-$ T cells

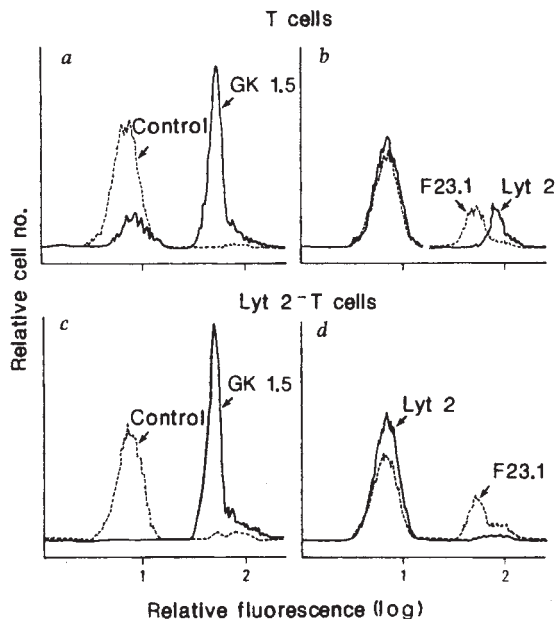


Fig. 1 Distribution of the F23.1 determinant on $\text{Lyt } 2^+$ and $\text{Lyt } 2^-$ T cells. Lymph-node cells of BALB.B adult female mice were treated with J11d⁹ B cell-specific monoclonal antibody (ascites fluid diluted 1:24) in *a* and *b* or with J11d plus AD415 (ref. 10) anti-Lyt 2 monoclonal antibody (ascites, 1:50) in *c*, *d*, followed by rabbit serum (1:20) as a source of complement. Surviving cells were recovered by Ficoll centrifugation and stained at 37°C for 20 min with either the anti-L3T4 rat monoclonal antibody GK1.5 (ref. 11) (ascites, 1:20) in *a*, *c*, or the monoclonal antibodies 53.6.72 (ref. 12) (rat anti-Lyt 2; ascites, 1:50) or F23.1 (mouse anti-T-cell receptor allotype; ascites, 1:100) in *b*, *d*; this was followed after two washes by incubation with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin for 20 min at room temperature. Cells were analysed for fluorescence on a Becton-Dickinson FACS IV. B-cell-depleted lymph-node cells were 70.0% L3T4^+ , 18.0% $\text{Lyt } 2^+$, 23.0% F23.1^+ . Such cells, additionally depleted of $\text{Lyt } 2^+$ T cells, were 95.5% L3T4^+ , 20.9% F23.1^+ and none was $\text{Lyt } 2^+$. Thus, F23.1 reacts with $\text{Lyt } 2^-$ as well as $\text{Lyt } 2^+$ T cells.

to respond under any circumstances, unselected and $\text{Lyt } 2^-$ T cells were stimulated in small-scale cultures with either F23.1-Sephadex beads or Con A in the presence of syngeneic spleen cells. The response of 5,000 $\text{Lyt } 2^-$ T cells to Con A (4,337 c.p.m.) was about one-third of the response of unselected T cells (11,884 c.p.m.), whereas the response to F23.1 represented only 1% of that of unselected T cells (375 c.p.m. versus 35,638 c.p.m.). This result argues that the $\text{Lyt } 2^-$ T cells, which were unresponsive to F23.1-Sephadex, were nevertheless viable and functional under appropriate activation conditions. $\text{Lyt } 2^+$ T cells were clearly necessary for the growth of clones in response to the immobilized anti-receptor antibody plus ConASN. To show that T cells of this subset were also sufficient to act as clonal precursors, purified $\text{Lyt } 2^+$ cells were prepared by positive selection using tissue culture dishes coated with the anti-Lyt 2 monoclonal antibody 3.168. The T cells adhering to such dishes, recovered by vigorous pipetting, comprised 2.0% B cells, 95.9% $\text{Lyt } 2^+$ and 0.7% L3T4^+ on restaining and FACS analysis. These positively selected $\text{Lyt } 2^+$ T cells were diluted in limiting numbers into microcultures containing F23.1-Sephadex beads and ConASN. Positive selection resulted in an increase in clonal precursor frequency from 1 in 96 (unselected) to 1 in 20 (positively selected); see Fig. 3b. As the frequency of responding cells (5.0%) exceeds the FACS frequency of B cells, T_h precursors or unstained non-B, non-T cells, none of these cell types can possibly be limiting in the culture system used here.

These studies show that $\text{Lyt } 2^+$ T_c precursors are directly stimulated by F23.1 antibody immobilized on Sephadex beads, and become responsive to soluble mediators present in ConASN

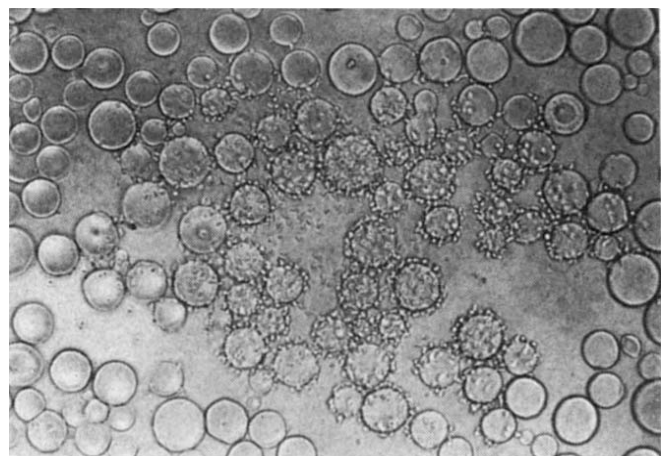


Fig. 2 Growth of T lymphocytes stimulated with F23.1 antibody coupled to Sephadex 4B beads. A 7-day culture was photographed using a Nikon Diaphot inverted microscope with a low-power ($\times 4$) objective. The field shows a cluster of activated cells adhering to antibody-coated beads. Culture conditions as in Fig. 3 legend.

which support their clonal expansion. $\text{Lyt } 2^-$, L3T4^+ T_h precursors, in contrast, are inert in this assay despite the fact that their antigen receptors carry the F23.1 determinant. In the experiments described so far, we used F23.1 antibody conjugated to beads at very high density (2.8 mg ml^{-1} in the coupling solution) and it is very difficult to imagine that greater ligand density is required to trigger L3T4^+ precursor T cells. However, to test the possibility that L3T4^+ T cells were unresponsive because of a non-physiological excess of receptor ligand, Sephadex beads were prepared with mixtures of F23.1 and bovine albumin to give a total protein concentration of 2.0 mg ml^{-1} in the coupling reaction, and a 1,000-fold range of F23.1 concentration (from 2 mg ml^{-1} to 2 $\mu\text{g ml}^{-1}$ in 10-fold steps). Unselected T cells were responsive to beads coupled to 2.0 and 0.2 mg ml^{-1} F23.1, but to no lower concentration. $\text{Lyt } 2^-$ T cells remained unresponsive at any concentration of F23.1 tested. At first sight, this absolute distinction is difficult to reconcile with findings in F23.1-activated bulk cultures, where a small proportion of viable L3T4^+ T blasts are reproducibly present after 4 days of culture (U.D.S. and M.J.B., in preparation); the precursors of such cells must either represent a rare $\text{Lyt } 2^-$ population (because they are undetectable in microcultures where T cells are limiting) or must instead carry $\text{Lyt } 2$ as well as the potential to express L3T4 . It is possible that these cells represent T_h precursors that are already activated *in vivo* and which are present in small numbers among resting T-cell preparations.

It is well known that T_c precursors may be induced to respond to helper signals following stimulation by a wide variety of cell types, which may be either viable or metabolically inactivated, whereas T_h precursors are triggered only by viable macrophage-like MHC class II-positive cells, known collectively as accessory cells. T cells of the $\text{Lyt } 2^+$ subset, but not of the L3T4^+ subset, may also be activated in bulk cultures by soluble Con A in the apparent absence of accessory cells⁵. Such activated $\text{Lyt } 2^+$ T cells express the IL-2 receptor⁶ and respond (by proliferation) to partially purified murine⁵ or pure recombinant human IL-2 (ref. 7). However, mitogenic lectins might activate T cells through pathways which do not involve the antigen receptor. For example, the mitogen phytohaemagglutinin (PHA) induces a rapid increase in intracellular Ca^{2+} concentration in human T cells, and this increase is inhibited by pretreatment of the T cells with antibody against the T11 molecule, suggesting that PHA activates T cells by binding to T11 (ref. 8). In addition, even purified $\text{Lyt } 2^+$ T cells in small-scale cultures could present Con A to each other and thereby deliver structural and soluble signals which are difficult to define.

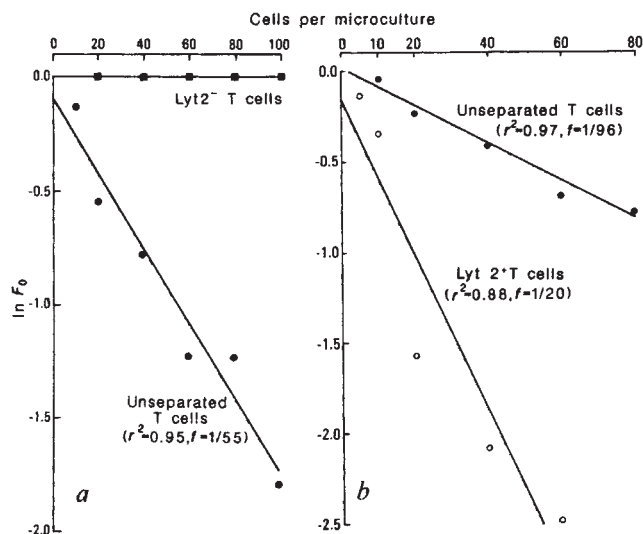


Fig. 3 *a*, Lymph-node 'T' cells from a single 6-week-old female BALB.B mouse (●), or such cells depleted of Lyt 2⁺ T cells (■), were prepared using monoclonal antibodies as described in Fig. 1 legend (such T cells are not highly purified with respect to macrophage contamination). Various mean numbers of cells from 10 to 100 were cultured in groups of 24 in half-area flat-bottomed microwells (Costar 3696) with 100 μ l of RPMI 1640 medium containing 10% (v/v) fetal calf serum (FCS; Flow Laboratories), 3×10^{-5} M 2-mercaptoethanol and glutamine, penicillin and streptomycin, plus 10% supernatant from rat spleen cells stimulated for 40 h with 2μ g ml⁻¹ Con A. Residual Con A was neutralized by the addition of 50 mM α -methyl mannose. Microwells contained $\sim 1,000$ Sepharose 4B beads (Pharmacia) CnBr-conjugated with F23.1 monoclonal antibody (2.8 mg ml^{-1} in coupling buffer) or BGG (2.7 mg ml^{-1}) as a control. After 7 days of culture at 37 °C in a 5% CO₂ incubator, microwells were either pulsed with 0.5 μ Ci ³H-TdR and harvested after 18 h (miniMASH, M.A. Bioproducts) for liquid scintillation counting (Tri-Carb 300, Packard), or scored for clonal growth by direct inspection using an inverted phase-contrast microscope (Diaphot, Nikon), as in this experiment. The figure shows least-squares regression lines of the natural logarithm of the fraction of microcultures that were negative ($\ln F_0$) on the average number of semi-purified T cells or Lyt 2-depleted T cells per culture. Precursor frequency (f) estimation is described in ref. 4. *b*, Semi-purified lymph-node T cells were prepared as described in Fig. 1 legend. Positively selected Lyt 2⁺ purified T cells were prepared by additional treatment with GK1.5 anti-L3T4 monoclonal antibody before the complement step, followed by panning¹³ on Falcon 1005 Petri dishes pre-coated by a 1-h incubation at room temperature with monoclonal antibody 3.168 (ref. 14) (anti-Lyt 2; ascites, 1:100). Non-adherent cells were removed by 10 cycles of washing by indirect pipetting with Hank's balanced salt solution (HBSS) containing 5% FCS, then adherent cells were collected (with $\sim 75\%$ recovery) by repeated direct pipetting. Such cells, stained for FACS analysis as described in Fig. 1 legend, were 95.9% Lyt 2⁺ T cells, 0.7% L3T4⁺ T cells, 2.0% B cells, with 1.4% not accounted for. Unselected semi-purified T cells or positively selected Lyt 2⁺ cells were cultured in limiting dilution with F23.1-Sepharose 4B beads and ConASN as described for *a*, and scored for clonal growth at 7 days.

By activating resting T cells using a ligand of the antigen receptor immobilized on Sepharose beads, these difficulties have been avoided. Thus, we conclude that cells bearing antigens plus MHC class I molecules need deliver no physical activation signal to resting T_c precursors beyond ligation of the antigen receptor; thereafter, they require only soluble mediators for clonal expansion. In contrast, T_h precursors require additional (undefined) signals as well as antigen receptor ligation. As the F23.1 antibody recognizes the antigen receptors of both T-cell subsets, the different and more exacting activation requirements of resting T_h precursors cannot be explained by their possession of a different specificity repertoire which is only engaged by antigens presented by MHC class II-positive accessory cells.

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Epstein-Barr virus-positive Burkitt's lymphoma cells not recognized by virus-specific T-cell surveillance

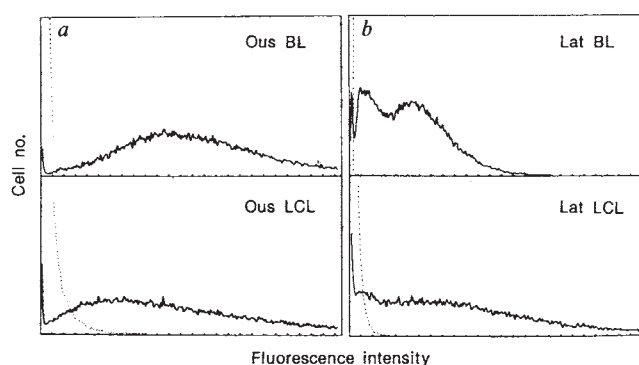
C. M. Rooney, M. Rowe, L. E. Wallace
& A. B. Rickinson

Department of Cancer Studies, Cancer Research Campaign Laboratories, University of Birmingham, Medical School, Birmingham B15 2TJ, UK

The pathogenesis of Epstein-Barr (EB) virus-positive Burkitt's lymphoma (BL) appears to involve the combined actions of (1) virus-induced B-cell proliferation, and (2) a rare chromosomal translocation juxtaposing *c-myc* and immunoglobulin gene loci in a single B cell; holoendemic malarial infection in some way facilitates the oncogenic process¹⁻³. Outgrowth of the EB virus-positive tumour suggests either breakdown or evasion of those immune controls, in particular cytotoxic T-cell responses against the virus-induced lymphocyte-detected membrane antigen LYDMA, which limit virus-infected B-cell numbers in healthy virus carriers⁴. Immunosuppression, such as that which malarial infection may induce^{5,6}, cannot itself be a sufficient explanation in this regard since our studies have identified a number of BL patients who retain detectable LYDMA-specific T-cell surveillance⁷. The present work shows that in many cases of virus-associated BL, the emerging malignant clone is insensitive to such surveillance. Several EB virus-positive BL cell lines, recently established *in vitro* and expressing the class I histocompatibility locus antigens (HLAs) which restrict cytotoxic T-cell function, were not killed by HLA-matched LYDMA-specific effector populations in assays where the EB virus-positive lymphoblastoid cell line (LCL), derived from normal B cells of the same patient, sustained high levels of lysis.

Table 1 gives details of the BL/LCL paired lines established for the purpose of this study either from endemic (high incidence, holoendemic malaria-associated) or from sporadic cases of EB virus-positive BL. Tumour cell lines arose by direct outgrowth from BL biopsies, whereas LCLs were obtained from normal B cells either spontaneously or by infection *in vitro* with the B95-8 strain of EB virus⁸; all lines were positive for the EB virus-coded nuclear antigen EBNA. In each case the BL cell line could be distinguished from its corresponding LCL by the presence of a specific t(8;14) chromosomal translocation and by a characteristic pattern of reactivity with a selected panel of monoclonal antibodies against B-cell-associated surface antigens⁹. In the present experiments, the target cells in use in cytotoxicity assays were regularly monitored by these criteria,

Fig. 1 Levels of class I HLA antigen expression on *a*, Ous BL and LCL cells, and *b*, Lat BL and LCL cells. Viable cells taken from exponentially growing cultures of the relevant cell line were exposed to the mouse IgG2a monoclonal antibody W6/32 (Seralab; 1:200 dilution of ascitic fluid) binding a common framework determinant on all class I HLA molecules. After incubation for 1 h at 4°C, the cells were washed twice in phosphate-buffered saline containing 5% normal goat serum (PBS-NGS) and exposed for a further 1 h at 4°C to a fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (Sigma; 1:100 dilution). After two further washes in PBS-NGS, the cells were resuspended in culture medium and the fluorescence labelling profile analysed using a fluorescence-activated cell sorter, cell number (vertical axis) being plotted against fluorescence intensity (horizontal axis). The level of background fluorescence, shown by cells exposed to the FITC conjugate alone, is in each case indicated by a dotted line.



particularly since some BL cell lines could progress *in vitro* to a more 'lymphoblastoid' phenotype without losing the karyotypic marker of tumour origin⁹ (see Table 1).

HLA typing, by standard serological tests, not only confirmed that each BL/LCL pair was indeed derived from a single patient (Table 1) but also made it clear that BL cells do express the class I HLA antigens which restrict target cell recognition by LYDMA-specific cytotoxic T cells⁴. Quantitative analysis of immunofluorescence labelling with the monoclonal antibody W6/32, specific for a framework determinant on all class I HLA molecules¹⁰, showed that most BL cell lines in the present panel were comparable to the corresponding LCL in their level of HLA antigen expression; a representative result from the Ous BL/LCL pair is shown in Fig. 1*a*. One exception was the Lat BL line, where expression was reduced compared with the paired LCL (Fig. 1*b*), but even in this case the BL cells remained clearly HLA antigen-positive and indeed were shown to be susceptible to allospecific cytotoxic T cells directed against class I HLA determinants¹¹.

These observations are important to bear in mind in considering the results of the main body of experiments where BL and LCL cells were compared for their sensitivity to EB virus-specific cytotoxicity. The effector cells used in these assays were polyclonal populations derived from healthy virus-immune donors by culturing peripheral blood mononuclear cells with appropriate numbers of autologous X-irradiated LCL cells, followed by short-term expansion of the T-cell response in interleukin-2-

conditioned medium; the EB virus-specific nature of this *in vitro* response is well documented^{4,12}. Only effector cell preparations displaying characteristic HLA-A and -B antigen-restricted lysis of LCL targets, and low lysis of EB virus-negative natural killer-sensitive targets such as the HSB2 and K562 cell lines¹², were selected for use in these experiments.

Figure 2 presents the results of one particular chromium release assay which illustrate a pattern seen on many occasions throughout this study. An EB virus-specific cytotoxic T-cell preparation from the virus-immune donor S.G. (possessing a strong A11-restricted component) clearly recognized the LCL targets from the A11-positive patients Wew 1 and Ous; as expected, such specific recognition could be blocked if the monoclonal antibody W6/32 was added at a concentration sufficient to pre-saturate class I HLA antigens on the target cell surface¹³. In the same assay, there was no significant lysis of the corresponding EB virus-positive BL cell lines Wew 1 (whether in early or late passage, the latter having progressed to a more 'lymphoblastoid' cell surface phenotype) and Ous.

Table 2 summarizes the cumulative data obtained from this type of experiment for all the BL/LCL pairs, many individual effector/target combinations having been tested on several occasions and always with concordant results. All three endemic and two of four sporadic BL cell lines were clearly insensitive to LYDMA-specific cytotoxicity by several different effector populations, each restricted through one or more of the HLA antigens on the BL cell surface. Significant lysis of the corresponding

Table 1 Characteristics of EB virus genome-positive BL/LCL paired lines

BL patient	Country of origin	Form of BL	Cell line type*	Karyotype	HLA antigen type	BL surface phenotype group†
Chep	Kenya	Endemic	BL	t(8; 14)	A2, A3; B7; Cw7; DR3, DR5	I
			LCL	diploid	A2, A3; B7; Cw7; DR3, DR5	
Eli	Kenya	Endemic	BL	t(8; 14)	A2, Aw23; B7, Bw22; ; DR3, DR7	I → II
			LCL	diploid	A2, Aw23; B7, Bw22; ; DR3, DR7	
Wew 1	New Guinea	Endemic	BL	t(8; 14)	A11, Aw24; B27, Bw62; Cw2, Cw4; DR2	I → II → III
			LCL	diploid	A11, Aw24; B27, Bw62; Cw2, Cw4; DR2	
Lat	La Réunion	Sporadic	BL	t(8; 14)	Aw28, Aw30; B18, Bw22; Cw2, Cw5; DR5, DR6	I → II
			LCL	diploid	Aw28, Aw30; B18, Bw22; Cw2, Cw5; DR5, DR6	
Ous	Algeria	Sporadic	BL	t(8; 14)	A11, Aw28; B27, Bw45; ; DR1, DR4	II
			LCL	diploid	A11, Aw28; B27, Bw45; ; DR1, DR4	
LS92	Algeria	Sporadic	BL	t(8; 14)	A2, Aw24; Bw44; Cw4, Cw5; DR1, DR7	II → III
			LCL	diploid	A2, Aw24; Bw44; Cw4, Cw5; DR1, DR7	
Lou	Algeria	Sporadic	BL	t(8; 14)	Aw31; B14, Bw44; Cw4, Cw5; DR1, DR6	II → III
			LCL	diploid	Aw31; B14, Bw44; Cw4, Cw5; DR1, DR6	

* BL cell lines were established by direct outgrowth from tumour biopsies whilst LCLs were established from normal B cells in blood samples either by spontaneous outgrowth (in the case of Ous LCL) or by experimental infection with the B95-8 strain of EB virus *in vitro*. Culture medium was RPMI 1640 supplemented with 2 mM glutamine, 100 IU ml⁻¹ penicillin, 100 µg ml⁻¹ streptomycin and 15% fetal calf serum from pre-selected batches.

† All BL cell lines were characterized within the first 10 *in vitro* passages and during subsequent (up to 100) passages for their cell-surface phenotype using a panel of monoclonal antibodies previously shown to be capable of revealing phenotypic differences between individual BL cell lines⁹. The antibodies were J5 (ref. 15) directed against the common acute lymphoblastic leukaemia antigen, 38.13 (ref. 16) directed against a 'BL-associated' antigen, and MHM6, AC2 (ref. 17), Ki-1 (ref. 18) and Ki-24 (ref. 19), all directed against different 'lymphoblastoid' antigens consistently expressed on all LCLs. The cell-surface phenotype of BL cell lines was classified according to the following patterns of antibody binding: group I, binding J5 and 38.13 only; group II, binding J5, 38.13 and one or more of the other antibodies; group III, binding MHM6, AC2, Ki-1 and Ki-24 only. As recently described⁹, all endemic BL cell lines display a group I phenotype in early passage, although certain lines may subsequently progress into group II or, rarely, into group III. Most sporadic BL cell lines (EB virus genome-positive) begin growth with a group II phenotype and many subsequently progress to group III. All LCLs display a group III-like phenotype.

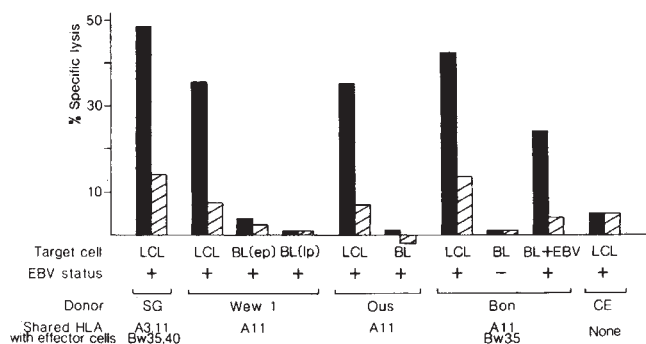


Fig. 2 Sensitivity of BL/LCL paired lines to EB virus-specific cytotoxic T cells. Results of a 5-h chromium release assay using effector cells from an EB virus-specific cytotoxic T-cell preparation generated *in vitro* from the virus-immune donor S.G. Target cells included (1) the autologous LCL from donor S.G., (2) the LCL and BL cell lines from patient Wew 1, the BL line being tested as both early(ep)- and late(lp)-passage cultures with group I and group III cell-surface phenotypes respectively (see Table 1), (3) the LCL and BL cell lines from patient Ous, (4) the LCL, EB virus-negative BL cell line and the virus genome-converted BL cell line (generated by experimental infection with B95-8-derived virus) from a non-virus-associated sporadic BL patient Bon, and (5) the LCL from an HLA-mismatched donor C.E. The EB virus genome status of each target cell line and its degree of HLA-A and -B antigen matching with the effector T cells is as shown. Results are expressed as the percentage specific lysis observed in a 5-h chromium release assay at an effector/target ratio of 5:1, either in normal medium (solid bars) or in the presence of the monoclonal antibody W6/32 (hatched bars) at a 1:200 dilution of an ascetic fluid preparation; this antibody concentration is sufficient to significantly block class I HLA antigen-restricted cytotoxicity through binding to class I HLA antigens on the target cell surface¹³.

LCL provided an essential positive control in these assays, indicating that effector/target combinations were indeed HLA antigen-matched; this was important since some BL patients were found to express variant HLA antigens which were serologically indistinguishable from the common equivalent in Caucasian populations but which had distinct T-cell restricting determinants (data not shown; compare ref. 14).

The inability of LYDMA-specific T cells to lyse the BL targets was not due to any inherent resistance of these targets to cytotoxicity *per se*¹¹; indeed, adding a lectin (phytohaemagglutinin) to circumvent the requirement for LYDMA-specific recognition in the above assays produced strong lysis of BL cells (data not shown). Nor do we feel that antigenic variation between the B95-8 laboratory strain of EB virus and strains naturally infecting the BL patients can adequately explain these findings, particularly since differential lysis of the LCL target was still observed in the one instance (Ous) where a spontaneously derived, rather than a B95-8 virus-transformed, LCL was available for comparison with the BL line. Rather, we favour the interpretation that in some, perhaps most, cases of EB virus genome-positive BL, the malignant cells do not express LYDMA, as this antigen is currently defined.

The BL cell phenotype itself does not appear to preclude LYDMA expression since two of seven BL cell lines were found to be positive for the antigen (Table 2) and an EB virus-negative BL line Bon (derived from a non-virus-associated sporadic case of BL) became LYDMA-positive following experimental infection with the virus *in vitro* (Fig. 2). In many cases, therefore, it seems that *in vivo* selection of a LYDMA-negative clone may well form a necessary additional step in the already complex pathogenesis of EB virus-associated BL. To what extent virus gene expression might be altered in such a clone remains just one of several intriguing questions raised by this work.

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Table 2 Sensitivity of BL/LCL paired lines to EB virus-specific cytotoxic T cells

BL patient	Effector T-cell donor*	Shared HLA antigens	EBV-specific lysis†	
			LCL	BL
Chep	GS	A2, A3, B7	++++	-
	CE	A2, B7	+++	-
	CF	A3, B7	++++	-
	SW	B7	++++	-
	SG	A3	++	-
Eli	GS	A2, B7	++++	-
	CE	A2, B7	+	-
	MH	A2	+	-
	SW	B7	++	-
	TM	B22	++++	-
Wew 1	CB	A11, Aw24, Bw62	++++	-
	SW	A11, Aw24	+++	-
	SG	A11	+++	-
	DW	B27	+++	-
Lat	MS	B18	++	-
	TM	B22	++++	-
Ous	SW	A11	++++	-
	SG	A11	+++	-
	SC	B27	++	-
LS92	MS	A2, Bw44	++++	++
	JB	A2, Bw44	++	++
	GW	A2, Bw44	+++	++
	VL	Bw44	+++	++
Lou	HD	Aw31, B14, Bw44	++++	++++
	VL	Bw44	+++	+

* EB virus-specific effector T-cell preparations were generated from healthy virus-immune donors by a 10-day co-cultivation of peripheral blood mononuclear cells with X-irradiated cells of the autologous B95-8 virus-transformed LCL (responder/stimulator ratio 40:1) followed by short-term expansion of the reactive T-cell population in interleukin-2-conditioned medium. Full details of these procedures are described elsewhere¹². Effector T-cell preparations were selected for these experiments only if they fulfilled all of the following criteria: (1) showing a class I HLA-restricted pattern of lysis against a panel of allogeneic LCL targets (2) showing low levels of lysis against a panel of EB virus genome-negative, natural killer cell-sensitive target cell lines¹², and (3) being matched with one or more of the BL patients in Table 1 through at least one class I HLA antigen.

† Summary of chromium release assay results obtained when EB virus-specific effector T-cell preparations were tested against paired BL/LCL target lines where effector and target cells shared the class I HLA antigens shown. In most cases, the particular effector/target combinations were tested on several occasions either using the same effector T-cell preparation or new preparations from the same virus-immune donor, and always with concordant results. To combine the results of successive assays, the level of target cell lysis observed was on each occasion expressed as a percentage of the lysis recorded in the same assay for the autologous LCL/effector T-cell combination tested at the same effector/target ratio (usually 5:1 or 10:1). Overall results for any one target could then be expressed as a relative percentage lysis in the following way: ++++ > 80%, +++ 60-80%, ++ 40-60%, + 20-40%, - < 10% of the level of autologous LCL lysis in the same assay. Absolute values of autologous LCL lysis varied between 40 and 80% specific chromium release.

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Nerve growth factor is a mitogen for cultured chromaffin cells

Laura E. Lillien & Philippa Claude

Neurosciences Training Program and Wisconsin Regional Primate Research Center, University of Wisconsin, Madison, Wisconsin 53715, USA

Nerve growth factor (NGF) is essential for the survival and differentiation of a number of neural crest derivatives, including sympathetic and sensory neurones¹⁻³. While early studies suggested that NGF might also have a mitogenic effect on these neurones^{4,5}, subsequent work has favoured the interpretation that NGF promotes cell survival or differentiation rather than proliferation^{6,7}. We have addressed the issue of a mitogenic effect of NGF using adrenal chromaffin cells, which are endocrine cells derived from the neural crest, and are closely related to sympathetic neurones. Adrenal chromaffin cells respond to NGF *in vitro* by expressing neuronal traits⁸⁻¹¹. We now report that NGF elicits a mitotic response in cultured chromaffin cells from young rats, and that this response is blocked by an antiserum to 2.5S NGF. The chromaffin cells that divide in response to NGF can subsequently become neuronal in the continued presence of NGF.

Unlike sympathetic neurones, adrenal chromaffin cells from rodents continue to exhibit significant mitotic activity after birth^{7,15-17}. Mitotic activity can also be observed *in vitro*. We grew chromaffin cells from 1-week-old rats as dissociated cells in the presence or absence of NGF for 1 week; at the end of this period *in vitro*, 1-2% of the chromaffin cells grown in the absence of NGF incorporated ³H-thymidine (³H-Thy) during a 24-h incubation (Figs 1, 2a, b), whereas 10-20% of the chromaffin cells in NGF-treated cultures incorporated ³H-Thy (Figs 1, 2c, d).

To confirm the identity of the labelled cells, we stained ³H-Thy-labelled cultures with an antiserum to tyrosine hydroxylase (TH) (Fig. 2i, j) to selectively label chromaffin or chromaffin-related cells rather than fibroblasts or glial cells (Fig. 2g, h). After 1 week in NGF, 10-15% of the TH-positive cells also had ³H-Thy-labelled nuclei (Fig. 2i, j). To verify that chromaffin cells that had incorporated ³H-Thy also went through cell division,

NGF-treated cultures were given a 6-h pulse of ³H-Thy; some were fixed immediately and some were fixed after various times of growth in unlabelled medium. The number of labelled chromaffin cells doubled by 24-48 h, indicating that most cells went on to divide following incorporation of ³H-Thy. At 24 h, 45% of the labelled cells appeared as doublets or quadruplets (Fig. 2e, f); by 48 h this proportion had increased to 60%, further indicating that the ³H-Thy incorporation reflects cell division.

NGF itself, rather than a contaminant in the NGF, appeared to be responsible for the stimulation of cell division. First, when we treated the cultures with NGF in the presence of an antiserum to 2.5S NGF, labelling of chromaffin cells was significantly reduced (Fig. 1). Second, dexamethasone, a synthetic glucocorticoid which antagonizes NGF-dependent neuritic outgrowth⁸⁻¹⁰, also antagonized the NGF-dependent enhancement of chromaffin cell division (Fig. 1). In addition, epidermal growth factor (EGF) failed to cause a comparable increase in ³H-Thy incorporation as would have been expected if EGF, as a contaminant of the NGF, caused the mitotic effect.

NGF appears to act as a mitogen, rather than simply permitting the survival of an actively dividing subpopulation of chromaffin cells that were NGF-dependent and would have died without it. Cultures were grown without NGF for 1 week (which resulted in a loss of 40-60% of the cells, compared with cultures treated with NGF), and were then switched to growth medium containing NGF. Only 1-2% of the chromaffin cells incorporated ³H-Thy in the control cultures. However, the proportion of ³H-Thy-labelled chromaffin cells still increased after the switch to NGF (Fig. 3). This indicates that cells competent to divide remained in the control cultures but did not divide until NGF was added. Similar effects were observed when NGF was added to cultures after 2 weeks of NGF deprivation.

What was the fate of the chromaffin cells that divided under the influence of NGF? It is well established that chromaffin cells can respond to NGF *in vitro* by extending neurites⁸; with prolonged exposure to NGF, these cells develop many characteristics of mature sympathetic neurones such as large spherical cell bodies and clusters of small vesicles⁹⁻¹¹. To determine whether the chromaffin cells that divide in NGF-treated cultures can also develop these characteristics, we exposed 1-week-old NGF-treated cultures to ³H-Thy for 24 h, then maintained them in unlabelled medium with NGF for 3 more weeks. We found

Fig. 1 Effect of NGF on the incorporation of ³H-thymidine (³H-Thy) in cultures of rat adrenal chromaffin cells. Cultures were grown in either control medium or medium containing NGF (100 ng ml⁻¹ 7S NGF) for 6 days. During the final 24 h, cultures were also exposed to ³H-Thy. Labelled cells were counted in wholemounts of cultures processed for autoradiography. Many more labelled chromaffin cells were present in cultures exposed to NGF than in control cultures ($P < 0.005$). This effect of NGF was blocked by an antiserum (Ab) to 2.5S NGF ($P < 0.005$), and by 10⁻⁵ M dexamethasone ($P < 0.005$). (Significance calculated using Student's *t*-test.)

Methods. Cultures of chromaffin cells were obtained from the adrenal medullae of 8-day-old Sprague-Dawley rats (King). Medullae were dissected free of cortical tissue, dissociated sequentially with 0.2% collagenase (Sigma type II) and 0.125% trypsin (Gibco) and grown in Medium 199 (Gibco) supplemented with 20% charcoal-stripped fetal calf serum (FCS; Sterile Systems or Gibco), 0.6% glucose and 100 µg ml⁻¹ gentamicin (Elkins-Sinn). The number of non-chromaffin cells was reduced by differential plating: cells were grown initially in 35-mm collagen-coated plastic tissue culture dishes for ~20 h, after which the chromaffin cells could be gently pipetted off the dish, leaving behind the more tightly adherent non-chromaffin cells. The purified chromaffin cells were then replated onto collagen-coated glass or plastic coverslips set into the bottom of tissue culture dishes to form a shallow well ~1 cm in diameter²¹; 10,000-15,000 cells were plated into each well. Over 90% of the cells in these cultures exhibited specific catecholamine histofluorescence or TH-immunoreactivity, even after 2 weeks *in vitro*. Of these, only 2-3 neurone-like cells per dish were observed during the first few days *in vitro*. The rest of the cells comprised primarily fibroblasts, which were readily distinguishable from chromaffin cells, and a few fusiform, phase-dark satellite cells²². Only characteristic chromaffin cells were counted. After 1 day *in vitro*, some cultures were exposed to NGF (100 ng ml⁻¹ 7S NGF; Collaborative Research), NGF plus 10⁻⁵ M dexamethasone (Hexadrol; Organon) or NGF plus an antiserum to 2.5S NGF (1:200 dilution; LAREF SA, Cademino, Switzerland). After 5 more days *in vitro* in these conditions, cultures were exposed to ³H-Thy (0.2-2 µCi ml⁻¹, 2 Ci mmol⁻¹; NEN) for 24 h, rinsed for 0.5-1 h in growth medium, fixed in 4% formaldehyde and 0.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7, and air-dried. In some cases cultures were exposed to ³H-Thy for only 6 h before being re-fed or fixed. Coverslips were coated with photographic emulsion (Kodak NTB2, diluted 1:1), exposed for 4-10 days at 4 °C, and developed in D-19 (Kodak). The proportion of ³H-Thy-labelled chromaffin cells (±s.e. of triplicate cultures) was determined by counting cells in two orthogonal strips in each culture dish (~8% of the total area of the well), using a Leitz inverted microscope with phase contrast and brightfield optics. Only heavily labelled chromaffin cells (see Fig. 2) having silver grains concentrated over nuclei were considered to be labelled.

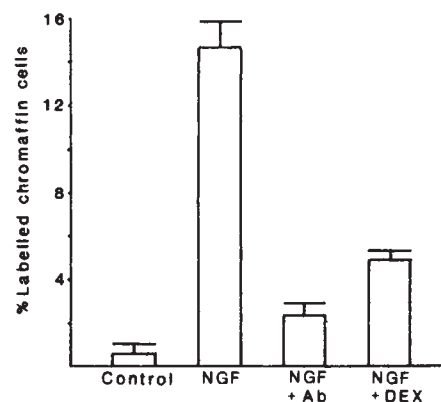
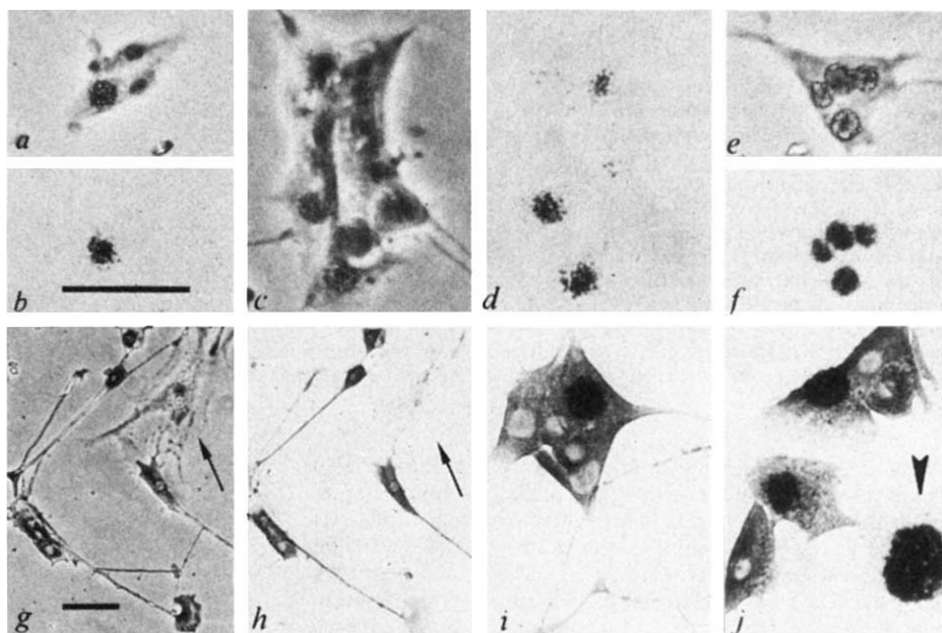
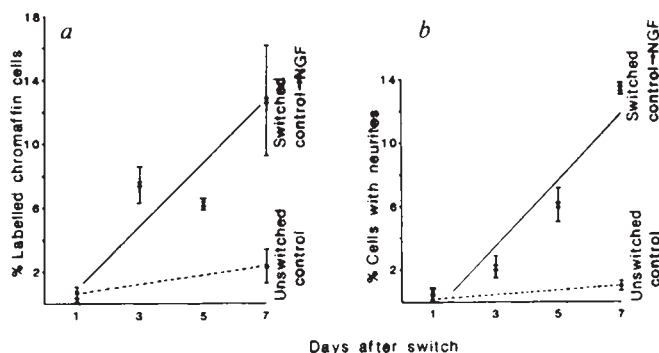


Fig. 2 *a-d*, Chromaffin cells from 8-day-old rats, after 1 week *in vitro*. Some have become labelled with ^3H -Thy during the final 24 h of culture after 1 week in control conditions (*a*, phase contrast; *b*, brightfield) or in NGF (*c*, phase contrast; *d*, brightfield). *e, f*, NGF-treated cultures were pulsed with ^3H -Thy for 6 h after 6 days *in vitro*, then rinsed, re-fed and fixed 48 h after the initiation of ^3H -Thy exposure. Approximately 60% of the labelled cells appear in doublets or quadruplets (*e*, phase contrast; *f*, brightfield). *g*, Phase contrast, and *h-j*, brightfield images of NGF-treated cultures stained with an anti-TH antiserum. The chromaffin cells are TH-positive while fibroblast-like cells, visible in *g* (arrow) are TH-negative (*h*). In *i* and *j*, cultures were exposed to ^3H -Thy for 24 h before staining with anti-TH. Of the TH-positive cells, 10–15% also have ^3H -Thy-labelled nuclei. The arrowhead in *j* indicates a ^3H -Thy-labelled fibroblast that is TH-negative. Scale bar in *b* equals 50 μm and applies to *a-f, i* and *j*; that in *g* equals 50 μm and applies to *h* also.



Methods. For TH immunocytochemistry, cultures were fixed in 4% paraformaldehyde, rinsed, permeabilized with 0.3% Triton X-100 and incubated overnight at 4°C with a rabbit antiserum to TH (1:500), supplied by T. H. Joh and G. N. Teitelman. Cultures were rinsed, then incubated with goat anti-rabbit IgG (1:50; Cappel: Cooper Biomedical, Inc., Malvern, Pennsylvania) and PAP (peroxidase-anti-peroxidase, 1:50; Polysciences Inc., Warrington, Pennsylvania). Peroxidase was developed with diaminobenzidine (40 mg per 100 ml; Sigma) for 4–6 min. Cultures were rinsed, air-dried and mounted in mineral oil. Some cultures were processed for autoradiography after drying (see Fig. 1 legend).

Fig. 3 NGF enhances the proportion of ^3H -Thy-labelled cells even in cultures that have been grown without NGF for 1 week. Cultures were grown in the absence of NGF (control medium) for a week, resulting in a loss of 40–60% of the cells compared with cultures grown in NGF. They were then switched to medium containing NGF. The proportion of cells that incorporated ^3H -Thy during a 24-h pulse was assessed at 1, 3, 5 and 7 days following the switch (*a*), as was the proportion of chromaffin cells that had formed neurites (*b*). Both responses increased, roughly in parallel, after addition of NGF. Bars represent s.e.m.



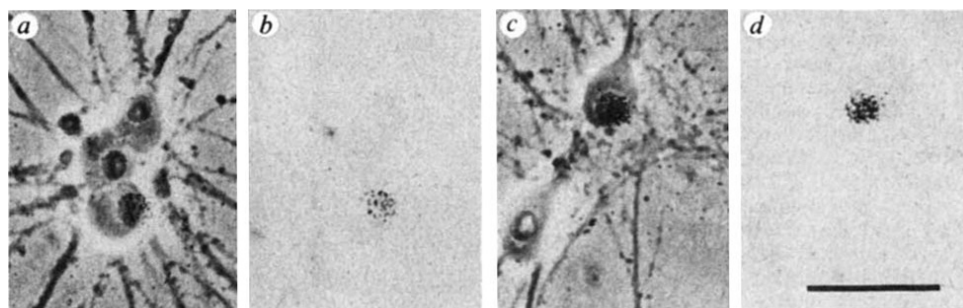
that 10–15% of the neurone-like cells in the 4-week-old cultures had labelled nuclei (Fig. 4), indicating that many of the cells that divided *in vitro* in response to NGF were indeed chromaffin cells that went on to differentiate into sympathetic neurone-like cells.

The results presented here indicate that NGF acts as a mitogen for cultured adrenal chromaffin cells from young rats. The mitotic response of these cells to NGF parallels their morphological response to NGF in several respects, suggesting that the enhancement of cell division is indeed an effect of NGF: (1) both responses to NGF are blocked by an antiserum to 2.5S NGF; (2) both responses are antagonized by dexamethasone¹⁸ at concentrations that are comparable to endogenous glucocorticoid levels in the adrenal gland⁸; (3) the maximal mitogenic effect occurs at the same concentration of NGF that maximally stimulates the formation of neurites *in vitro* (our unpublished observation); (4) the effect of NGF on cell division occurs with about the same delay, 2–3 days, as its effect on the formation of neurites¹⁸ (Fig. 3); and (5) neither response is mimicked by EGF, which is a mitogen for pheochromocytoma (PC12) cells^{12,13}, a tumour cell line related to the sympatho-adrenal system.

NGF has generally been reported to promote the differentiation of transformed cell lines of presumed neural crest origin, but there are some instances in which NGF has also been reported to enhance cell number or cell division among these cells. These effects were seen in variant clones of PC12^{12,13}, neuroblastoma¹⁴ or melanoma cell lines (M. Bothwell, personal communication), and in most cases were observed only at low concentrations of serum (that is, 1–2% rather than 15–20% serum; ref. 12 and M. Bothwell, personal communication) or only transiently following administration of NGF¹³. In our primary cultures of chromaffin cells the effect of NGF on cell division persisted for more than a week, even in the presence of 20% serum. In another study, NGF-treated PC12 cells were reported to incorporate ^3H -Thy into DNA without necessarily undergoing cell division, so that many became polyploid¹⁹. We verified that the chromaffin cells in these primary cultures do in fact go on to divide following incorporation of ^3H -Thy.

It is perhaps surprising that a direct mitogenic effect of NGF has not been observed before, in primary cultures or *in vivo*. For example, while adrenal chromaffin cells can respond to exogenous NGF *in vivo* by expressing neuronal characteristics²⁰, a mitogenic effect of NGF on these cells has not been reported.

Fig. 4 Chromaffin cells that divide in response to NGF can go on to differentiate into neurone-like cells. NGF-treated cultures were exposed to ^3H -Thy for 24 h on day 6 *in vitro*, when ~15% of the cells incorporated ^3H -Thy, and were then grown for another 3 weeks in unlabelled medium in the presence of NGF before being fixed and processed for autoradiography. At 4 weeks, 90% of the cells are indistinguishable from sympathetic neurones; in addition to the formation of neurites seen early during NGF treatment, they now also have large spherical cell bodies and clusters of synaptic vesicles, and they have become postmitotic. 10–15% of these neurone-like cells are labelled as a result of exposure to ^3H -Thy on day 6 *in vitro*. *a, c*, Phase contrast; *b, d*, brightfield optics. Scale bar, 50 μm .



In view of the present results, it seems possible that NGF also acts as a mitogen for other neural crest derivatives at early stages of their development. In fact, chromaffin cells from young rats appear to have retained some of the characteristics one might attribute to a sympatho-adrenal precursor cell, although they already express a chromaffin cell phenotype: they are developmentally pluripotent, in that they can still express either a neuronal or a chromaffin phenotype, and they are not yet postmitotic. The mitotic response of chromaffin cells to NGF *in vitro* may thus reflect a potential mitogenic effect of NGF on precursor cells of the sympatho-adrenal lineage *in vivo*.

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Expression of *engrailed* in the parasegment of *Drosophila*

Philip Ingham*, Alfonso Martinez-Arias, Peter A. Lawrence & Ken Howard*

* Imperial Cancer Research Fund, Burtonhole Lane, Mill Hill, London NW7 1AD, UK
MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

The first hint of segmentation in the *Drosophila* embryo is the appearance of shallow transverse grooves that divide the main part of the germ band into 14 uniform domains¹. These grooves are conspicuous in the 5–7-h-old embryo when viewed in the scanning electron microscope² and can also be seen in longitudinal sections. Since their discovery in 1950, the domains delineated by the grooves have been thought to be segments^{1,2}, but now several lines of evidence suggest that they are 'parasegments'—units consisting of a posterior compartment³ of one segment and an anterior compartment of the next^{4,5}. If this is the case, the posterior compartments would lie in the anterior parts of the domains delineated by the grooves. By using the *engrailed* gene product as a marker, we have now mapped the posterior compartments and report here that they are indeed located in the anterior quarter of the domains and that their anterior borders coincide with the grooves, establishing that the domains are parasegments.

In the ectoderm each segment consists of one anterior plus one posterior compartment^{3,6} and only the cells of posterior compartments require the *engrailed* gene for normal pattern formation^{7–11}; transcription of the *engrailed* gene is therefore a marker for cells belonging to posterior compartments. Specific

probes for *engrailed* transcripts have now been made^{12,13} and *in situ* hybridization has shown that *engrailed* expression is limited to posterior cells in sections of 8–13-h embryos¹³ and in whole mounts of imaginal disks¹³. For embryos, this method relies on frozen sections and unfortunately these nearly obliterate the grooves. Here we have used material embedded in paraffin wax^{14,29} to study the position of the posterior compartments in relation to the grooves and other landmarks.

Figure 1 shows that *engrailed* transcription is confined to the anterior-most quarter of the metamere defined by the grooves. The metameres are therefore parasegments and will be referred to as such throughout. Anterior to the midpoint of the parasegment and lateral in the embryo, there are tracheal pits which were thought by Keilin to arise at the segment boundaries¹⁵. If this were the case, one would expect the anterior half of the pit to express *engrailed* and the posterior half not to; this is indeed what we observe (Fig. 1*b, c*).

There are more than 14 regions of *engrailed* transcription^{12,13}. Anterior to the first parasegment (which corresponds to the posterior mandibular compartment⁴) but posterior to the stomodaeum, there is another band, which may correspond to part of the premandibular segment^{16,17}. Anterior to the stomodaeum is a further band that is located in the labrum. Posterior to the 14th parasegmental stripe (corresponding to the posterior compartment of A8; ref. 4) there is a band near the anus; additionally, the dorsal part of the hindgut and portions of the malpighian tubules are labelled (not the midgut, as stated in refs 12, 13) (see Figs 1, 2).

When the germ band shortens between 9 and 11 h, the epidermis is thrown into deep folds; *in situ* hybridizations at this stage show that these folds define segments, the deepest point of the infolding being within 1–2 cells of the segment boundary. When seen in longitudinal sections, each segment is ~12–16 cells across and of these cells, 3–4 express transcripts homologous

Fig. 1 Localization of *engrailed* transcripts at the extended germ-band stage of embryogenesis. Sections are oriented with their dorsal side uppermost and anterior to the left. **a**, A medial longitudinal section (see **c** for illustration of the plane of section) of the posterior region of the embryo, showing the ectodermal grooves (indicated by black dots) which delineate the parasegments. The grains are associated with about one-quarter of the cells located in the anterior part of each parasegment (this is particularly clear in parasegment 7 of this specimen). The anterior-most cell that is expressing *engrailed* is very close to the centre of the groove. Scale bar, 20 μ M. **b**, A lateral longitudinal section through an embryo at a similar stage to that shown in **a**. This plane of section passes through the tracheal pits (p) (see **c**). The location of grains indicates that cells which contribute to the anterior (but not the posterior) wall of each pit express *engrailed*. Scale bar, 20 μ M. **c**, Schematic representation of part of an extended germ-band-stage embryo, illustrating the planes of section shown in **a** and **b** and their relationship to the ectodermal grooves and tracheal pits. The shaded regions indicate the predicted localization of *engrailed* transcripts in the posterior but not the anterior (A) compartments, based on the assumption that the grooves correspond to parasegmental boundaries. This distribution corresponds closely to that observed in **a** and **b**. If the regions demarcated by the grooves were equivalent to segments, the regions of *engrailed* expression would have been located posteriorly within each unit, and remote from the tracheal pits. **d**, Dark-field image of a medial longitudinal section through an embryo similar to that shown in **a**. In addition to the 14 stripes of label in parasegments 1–14, there are two more anterior and two more posterior regions of *engrailed* expression. The most anterior region is located in the labrum (l); between this and parasegment 1 is a region which probably corresponds to the intercalary or premandibular segment (m). Posterior to parasegment 14, *engrailed* is expressed in the anal region (a) and hindgut (h). Scale bar, 100 μ M.

Methods. Embryos were fixed in formaldehyde (4%) and embedded in paraffin wax. Sections (6 μ M) were hybridized and washed essentially as described previously^{14,26,27,29}. The probe was ³⁵S-labelled single-stranded RNA synthesized in the SP6 system from a 0.9-kilobase *engrailed* genomic *Eco*RI fragment derived from the clone pF7036 (ref. 12); although this includes sequences homologous to the homoeo box and to the *engrailed-related* gene²⁸, the pattern of transcription it detects in early embryos is identical to that revealed by a probe derived from sequences in the *engrailed* complementary DNA^{12,13}. Slides were developed after a 14-day exposure at 4 °C.

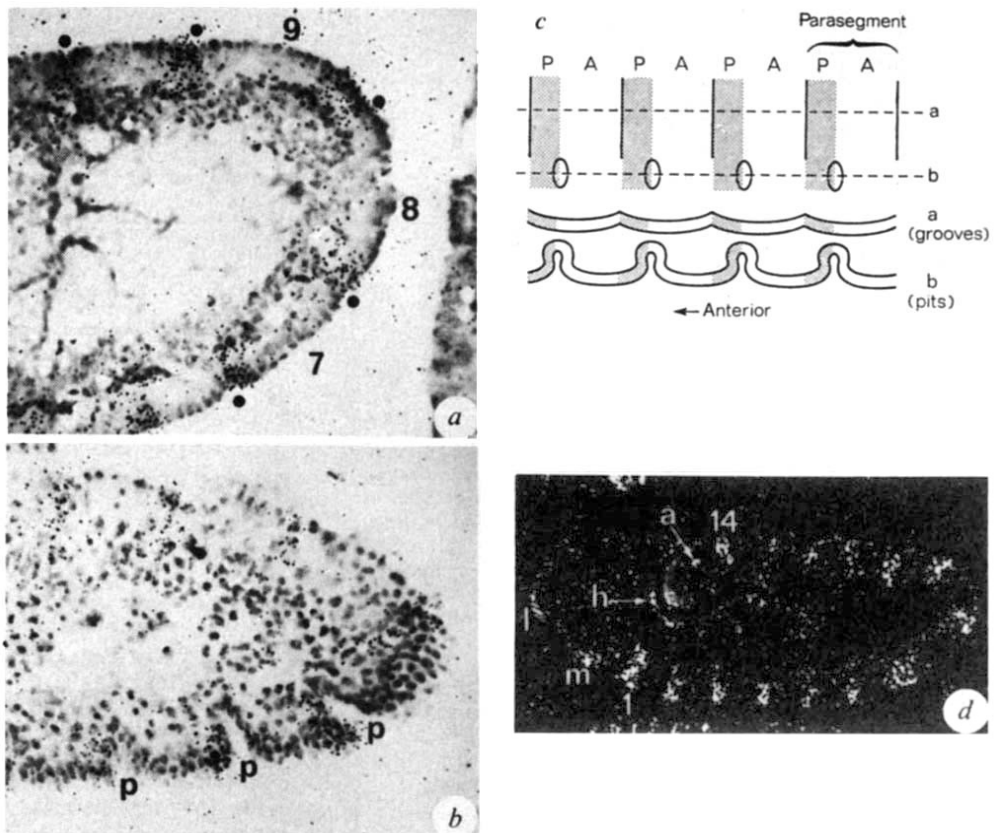
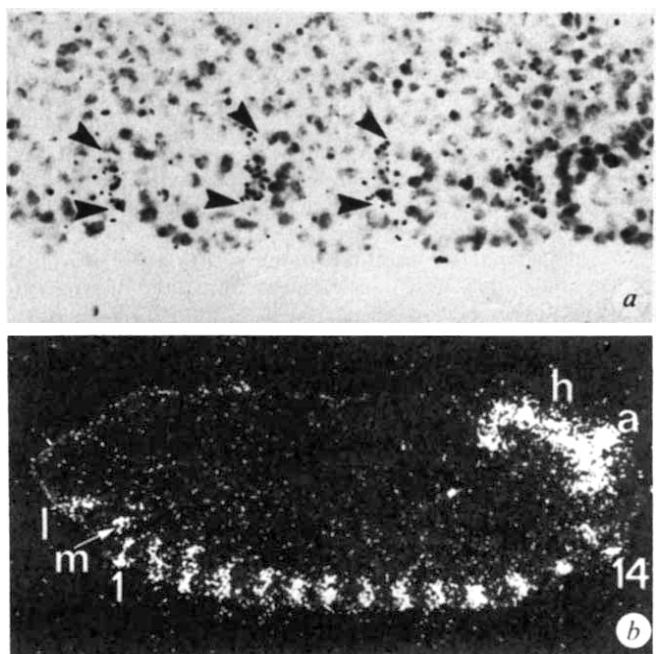


Fig. 2 *In situ* hybridization of the *engrailed* probe to the shortened germ band. **a**, Detail of the ventral region of a 10-h embryo in which the epidermis is thrown into deep folds that are approximately segmental; 3–4 cells bind the *engrailed* probe in the posterior part of each segment (~12–16 cells). Arrowheads indicate the limits of *engrailed* expression and therefore the boundaries between the anterior and posterior compartments. Scale bar, 20 μ M. **b**, Medial longitudinal section of a 10-h embryo, showing the same labelled zones as shown in Fig. 1d (same abbreviations). Scale bar, 100 μ M. Hybridization conditions were as described in Fig. 1 legend, except that the probe in **a** was labelled with ³H.



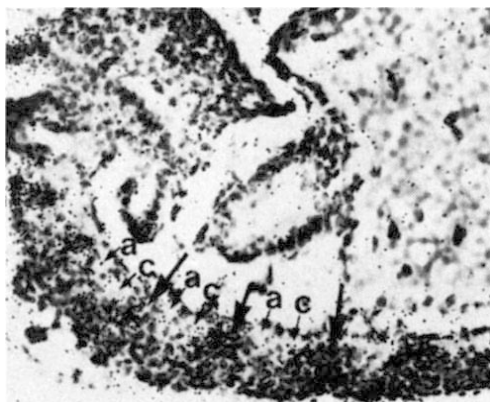


Fig. 3 Expression of *engrailed* in the CNS. Medial longitudinal section of a 12-h embryo, showing reiterated zones of hybridization in part of the ventral nerve cord. Connectives can be seen in cross-section; connectives labelled a and c (ref. 23 and M. Bate, unpublished) are close together, separated by only a single row of neuronal cell bodies. Between c and a there are more cell bodies, some of which express transcripts which hybridize to the *engrailed* probe and therefore appear to belong to posterior compartments. This pattern of labelling suggests that the segment boundaries lie near to a line drawn halfway between c and a (arrows), which is in agreement with, and provides independent support for, previous conclusions (ref. 23 and M. Bate, unpublished). Scale bar, 20 μ M. The probe and hybridization conditions were as described in Fig. 1 legend.

to the *engrailed* probe (Fig. 2). At early gastrulation, when each segment is about 3–4 cells across¹⁸, only one posterior cell expresses *engrailed*¹³. Lineage studies have estimated that all epidermal cells divide about twice between the blastoderm stage and 10 h^{19,20}, which is in good agreement with these observations.

As the embryonic ectoderm generates epidermis and neurones^{1,21}, one would expect the epidermis and developing central nervous system (CNS) to be compartmentalized concurrently. It was therefore surprising when Kornberg *et al.*¹³ reported expression of *engrailed*⁺ in the epidermis but not in the CNS, particularly as a requirement for *engrailed*⁺ in the CNS has been reported²². The better preservation achieved in wax sections here reveals clear expression of *engrailed*⁺ in the CNS (Fig. 3), and provides an independent mark for the position of the segment boundary that confirms earlier estimates (ref. 23 and M. Bate, unpublished).

Expression of *engrailed* is detectable in the mesoderm soon after gastrulation but it fades away rapidly^{12,13}. For several reasons, discussed elsewhere^{23–25}, we do not consider the mesodermal parasegments to be divided into anterior and posterior compartments and therefore suspect that the fleeting *engrailed* transcription makes no contribution to the genetic address of the mesoderm.

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Post-transcriptional control of *myc* and *p53* expression during differentiation of the embryonal carcinoma cell line F9

Carola Dony, Michael Kessel & Peter Gruss

Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), Im Neuenheimer Feld 282, D-6900 Heidelberg, FRG

Teratocarcinoma cells provide us with a model system for the study of differentiation and development^{1–3}. One of the best characterized cell lines, the embryonal carcinoma stem cell line F9, differentiates after treatment with retinoic acid (RA) and dibutyryl cyclic AMP into parietal endoderm⁴. This differentiation process is accompanied by the induction of several genes, for example, those encoding collagen IV, plasminogen activator and intermediate filaments like laminin^{4–6}. In contrast, a marked reduction of stable messenger RNA has been observed for the gene encoding *p53* and for *c-myc*^{7,8}. Both cellular oncogenes seem to be involved in the regulation of cellular proliferation and neoplastic transformation^{8–12}. For growth-arrested 3T3 fibroblasts, growth-factor-induced changes of *myc* RNA are controlled at the level of transcription¹³. In contrast, F9 cells provide a differentiation system in which cells are able to change from a tumorigenic state into non-dividing, non-tumorigenic endodermal cells⁵. The latter process enabled us to study the regulation of *myc* and *p53* genes in the same cells at different stages of growth, tumorigenicity and differentiation. Here we report that down-regulation of stable *myc* and *p53* RNA during irreversible differentiation of F9 cells occurs at the post-transcriptional level. Using an *in vitro* nuclear transcription assay¹⁴, we found that the polymerase II density on both genes remains constant during differentiation. In agreement with this interpretation, we detected *myc* RNA as stable transcripts in differentiated F9 cells after treatment of the cells with cycloheximide. The post-transcriptional regulatory mechanisms controlling *p53* and *myc* stability follow different kinetics. Whereas the down-regulation of *myc* seems to be an early event of F9 differentiation occurring within the first 24 h, the post-transcriptional regulation of *p53* occurs at a later stage (two to three days), possibly as a consequence of cell cycle changes.

In order to analyse whether control is exerted at the transcriptional level during F9 differentiation, we measured the transcription rate of *myc* and *p53* mRNA in isolated nuclei at various times after RA and cAMP addition by the nuclear run-on transcription method. In this assay, transcripts that are initiated *in vivo* are faithfully elongated *in vitro* using ³²P-labelled ribonucleotide triphosphate. The hybridization of labelled RNA to complementary DNA immobilized on nitrocellulose filter paper reveals a measure of the level of transcription at the time of cell lysis, because new RNA transcripts are not initiated *in vitro*^{14–16}. Analysis of the results of the nuclear run-on assay at various times shows that the rates of transcription of *myc* and *p53* do not change during the differentiation of F9 cells (Fig. 1). In control experiments the β -actin transcription rate was also constant while the gene for α -fetoprotein, which is typical of visceral

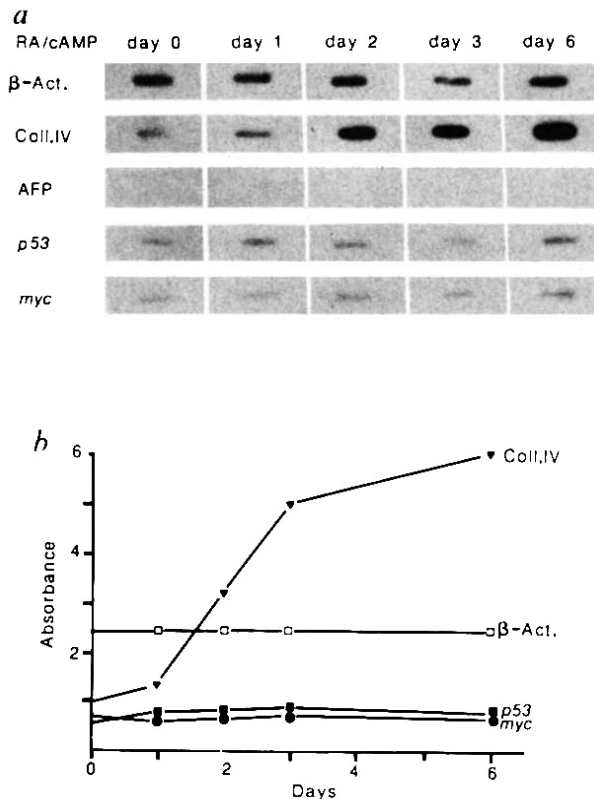


Fig. 1 Transcriptional analysis of *p53*, the genes *c-myc* and for β -actin, collagen IV and α -fetoprotein (AFP) during the differentiation of F9 cells into parietal endoderm. **a**, Autoradiograms of the hybridization of 32 P-labelled run-on transcripts to cDNA clones of *p53*, *c-myc*, β -actin, collagen IV and AFP bound to nitrocellulose. Nuclei were isolated from F9 stem cells (day 0) and from F9 cells at different times (1, 2, 3 and 6 days) after induction with RA and cAMP. The relative amount of labelled nuclear run-on transcripts hybridized to *p53* and *c-myc* DNA was determined by quantitative densitometric scanning of autoradiographs (**a**). β -actin was used as the denominator and AFP as a negative control.

Methods. For cell culture, F9 cells (provided by S. Strickland) were grown in gelatinized 150-cm² tissue culture flasks in Dulbecco's modified Eagle's medium (DMEM) containing 10% serum. For induction, cells were exposed to 5×10^{-7} M RNA and 10^{-3} M cAMP as described by Strickland *et al.*⁵. For the nuclear run-on transcription assay, nuclei were prepared for *in vitro* transcription as described by Schibler *et al.*²⁶, but with variations. Three confluent 150-cm² tissue culture flasks of F9 cells were rinsed with ice-cold phosphate-buffered saline, scraped into the same buffer and pelleted at 1,500 r.p.m. The cell pellet was resuspended in 10 ml of lysis buffer²⁷ containing 0.05% Nonidet P-40 and lysed by repipetting the cell suspension 10–15 times with a 10-ml plastic pipette. Nuclei were pelleted at 1,500 r.p.m. for 4 min and resuspended in 10 ml of lysis buffer containing $10 \mu\text{g ml}^{-1}$ RNase A²⁸. After 30 min on ice the nuclei were centrifuged (1,500 r.p.m., 10 min) through a 3.5-ml cushion of 30% (w/w) sucrose in lysis buffer. The pellet was resuspended in 0.2 ml nuclei storage buffer²⁸ at a concentration of 10^8 nuclei ml⁻¹ and stored at -70°C until use. Nuclei (2×10^7) were thawed for the run-on transcription assay and incubated for 25 min at 26°C in 0.2 ml of 70 mM KCl, 5 mM MgCl₂, 2.5 mM dithiothreitol (DTT), 0.1 mM EDTA, 0.4 mM each of ATP, GTP and CTP, 0.03 mM UTP and 200 μCi [α - 32 P]UTP (600 Ci mmol⁻¹; NEN). Purification of labelled RNA was done as described elsewhere by Groudine *et al.*¹⁴. 5×10^6 c.p.m. of labelled RNA was hybridized for 4 days to cDNA clones immobilized on nitrocellulose filters (10 μg plasmid DNA per slot) as described by Maniatis *et al.*^{29,30}. Filters were washed twice in $2 \times \text{SSC}$ (1 M NaCl, 0.03 M tri-sodium citrate) at 60°C and treated for 30 min at room temperature with RNase A ($10 \mu\text{g ml}^{-1}$ in $2 \times \text{SSC}$). Then the filters were washed again at room temperature in $0.1 \times \text{SSC}$ for 30 min. Plasmids were provided by M. Oren (*p53*; ref. 31), R. Müller (*c-myc*; ref. 32), S. Strickland (collagen IV; ref. 33), B. Paterson (β -actin) and S. Tilghman (AFP; ref. 34).

endoderm, remained inactive throughout parietal endoderm differentiation. In contrast, the transcription rate of the collagen IV gene, which is active in parietal endoderm cells, was stimulated approximately 10-fold during the differentiation of F9 cells, as shown previously by Gudas *et al.*¹⁷. Treatment with a low concentration of α -amanitin abolished detectable transcription in the nuclear run-on assay (data not shown), demonstrating

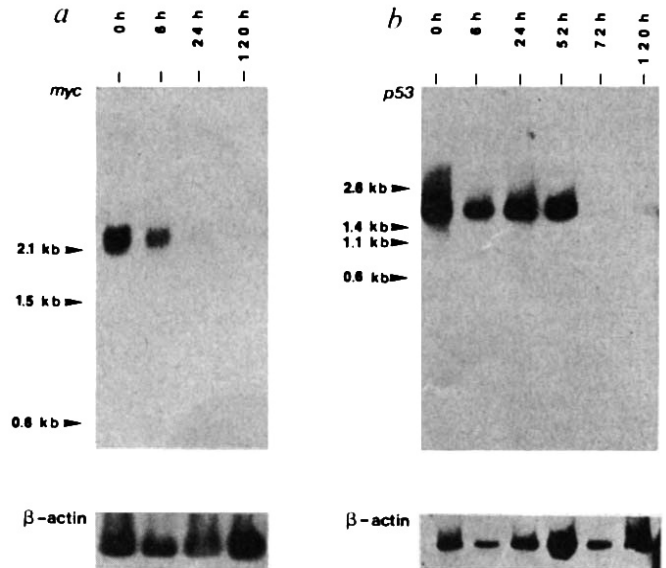


Fig. 2 Kinetics of *c-myc* and *p53* transcription in F9 cells after induction with RA/cAMP. **a**, Poly(A)⁺ RNA from F9 cells isolated at 0, 6, 24 and 120 h after treatment with RA (5×10^{-7} M) and cAMP (10^{-3} M) was hybridized with 32 P-labelled *c-myc* RNA by Northern blotting. **b**, Hybridization of poly(A)⁺ RNA isolated from F9 cells at 0, 6, 24, 52, 72 and 120 h after induction, hybridized to a nick-translated probe of *p53* DNA. Autoradiograms of control hybridizations of the Northern blots (**a** and **b**) with β -actin DNA are shown at the bottom.

Methods. F9 cells were grown and induced for differentiation as described in Fig. 1 legend. RNA was isolated by the guanidinium thiocyanate method described by Chirgwin *et al.*³⁵. Poly(A)⁺ RNA was selected by chromatography over oligo(dT)-cellulose. RNA (6 μg) was gel-electrophoresed, blotted onto Gene Screen plus (NEN) and hybridized to nick-translated plasmids containing *p53* or *c-myc* sequences. Control hybridization was with a β -actin probe. For hybridization, Northern blots were incubated for 24 h with nick-translated probes in 50% formamide, 1.0 M NaCl, 1% SDS and 100 $\mu\text{g ml}^{-1}$ denatured salmon sperm DNA. Blots were washed twice in $2 \times \text{SSC}$ at room temperature and twice in $2 \times \text{SSC}$ and 1% SDS at 60°C , with constant agitation.

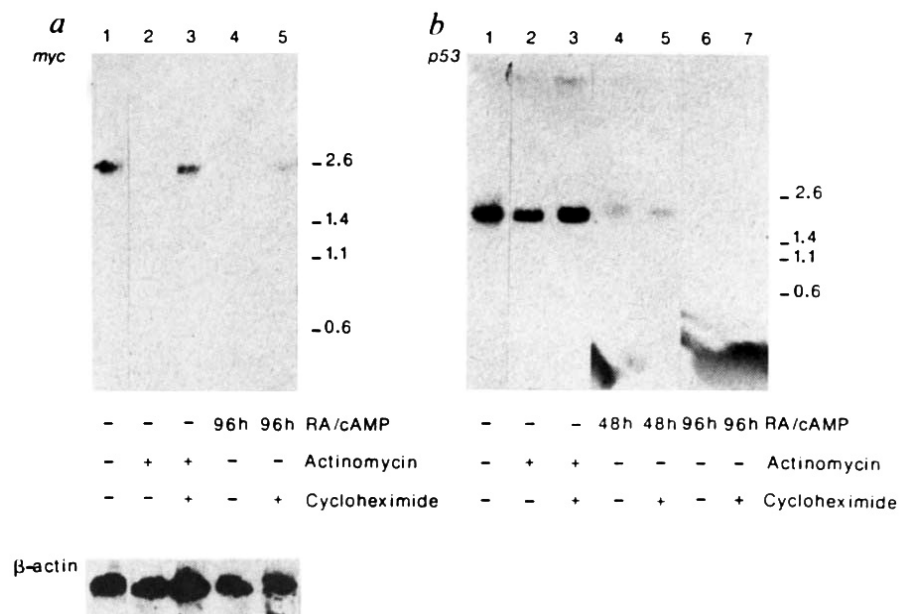
that transcription is carried out by polymerase II. In a parallel set of experiments, run-on transcription was analysed in digitonin-permeabilized whole cells¹⁸. The data corroborated the results obtained with isolated nuclei (not shown).

The apparent transcription of both *p53* and *myc* with equal efficiency throughout F9 differentiation led us to study the amount of the respective stable transcripts at various times after induction. For this purpose the time course of expression of stable transcripts was determined by Northern blot analysis of F9 RNA isolated at different times after induction with RA/cAMP. The differentiation-associated down-regulations of *c-myc* and *p53* follow different kinetics (Fig. 2). Reduction of *myc* RNA is a very early event in the differentiation process. Just 6 h after induction, stable *myc* RNA is significantly decreased, with only a minute amount detectable after 24 h. In contrast, a reduction of *p53* RNA can be detected after only 72 h of differentiation and a visible amount of RNA is still present after 120 h.

The apparent discrepancy between the data obtained by run-on and Northern analyses can be resolved if, during the process of differentiation, *myc* and *p53* expression is not regulated at the level of initiation but rather by a post-transcriptional mechanism, possibly by a mechanism affecting the half-life of the transcripts. We treated F9 cells at different stages of differentiation with actinomycin and cycloheximide. After inhibition of RNA synthesis with actinomycin for 2 h, the level of stable *myc* RNA in F9 stem cells was significantly decreased (Fig. 3a). This observation agrees with previous data¹⁹ which indicate that the half-life of *myc* RNA is less than 2 h. Pretreatment for 30 min with an inhibitor of protein synthesis (cycloheximide), followed by addition of actinomycin for 2 h, prevented this effect (Fig. 3a). In other words, addition of cycloheximide prevents degradation of mRNA by extending its half-

Fig. 3 Transcription of *c-myc* and *p53*-specific RNA in F9 stem cells and differentiated F9 cells after treatment with actinomycin and cycloheximide. **a**, Northern blot of poly(A)⁺ RNA from F9 cells hybridized to a nick-translated probe of *c-myc* DNA. Lane 1, RNA from untreated F9 cells; Lane 2, RNA from actinomycin-treated stem cells; lane 3, cells treated with actinomycin and cycloheximide; 4, RNA from F9 cells induced with RA/cAMP for 96 h; 5, RNA from the same cells as in lane 4, treated with cycloheximide. An autoradiogram of a control hybridization of the same blot with β -actin DNA is shown also. **b**, Hybridization of nick-translated *p53* DNA to F9 cellular A⁺ RNA. RNA was isolated from F9 cells treated in the following way: lane 1, F9 stem cells; lane 2, F9 cells after actinomycin chase; lane 3, F9 cells after actinomycin and cycloheximide treatment; 4, F9 cells induced with RA/cAMP for 48 h; 5, the same cells as shown in lane 4, after actinomycin chase; 6, F9 cells differentiated for 96 h after induction with RA/cAMP; 7, the same cells as in lane 6, after cycloheximide treatment.

Methods. F9 cells were grown as for Fig. 1. For the actinomycin chase, cells were grown for 2 h with 5 μ g actinomycin D per ml of medium. Cycloheximide treatment was for 2 h, at 10 μ g per ml of medium. For combined treatment, cycloheximide was added 30 min before actinomycin. After isolation of total RNA by the guanidinium thiocyanate method³⁵, poly(A)⁺ RNA was selected by oligo(dT)-cellulose chromatography. For Northern analysis, 6 μ g of A⁺ RNA separated on an agarose gel was transferred to Gene Screen-plus and hybridized to nick-translated DNA probes. Hybridization and washing as for Fig. 2.



life. Cycloheximide treatment of differentiating F9 cells also resulted in reappearance of stable *myc* RNA, as analysed by the Northern technique (Fig. 3a). Thus use of a different experimental approach confirmed the results obtained by nuclear run-on analysis. It seems reasonable to conclude that the primary transcripts detected by run-on analysis can be stabilized by protein synthesis inhibitors, resulting in an increase of stable *myc* transcripts which now become detectable in the differentiated cell.

In contrast to *myc* RNA, the amount of *p53* transcripts remains constant after treatment with actinomycin or cycloheximide (Fig. 3b). Even after 6 h of treatment with actinomycin, the amount of stable *p53* RNA was not significantly decreased (data not shown). Therefore *p53* RNA seems to have a longer half-life than *myc* RNA.

In addition to the F9 system, a down-regulation of stable *myc* mRNA has been previously described for HL 60 cells after treatment with vitamin D metabolite²⁰ and for Daudi cells after interferon treatment²¹. These chemicals inhibit cellular mitosis, resulting in a reduction of growth activity. The opposite reaction, mitogenic stimulation, has been demonstrated to induce *myc* and *p53* expression⁸⁻¹⁰. All these results lead to the hypothesis that *myc* and *p53* are proliferation-associated genes. Although for Daudi cells reduction of *myc* expression, which is due to a G₀/G₁ arrest, has been reported to be transcriptionally regulated²², evidence from other groups points to a post-transcriptional mechanism²³. No reason for this apparent discrepancy is yet known. Another transcript, tubulin mRNA is also down-regulated at the post-transcriptional level during differentiation²⁴. Our results with the F9 differentiation system indicate that cells which enter the differentiated state of parietal endoderm regulate the proliferation-associated genes *p53* and *myc* at the post-transcriptional level. These processes may belong to common phenomena essential for the maintenance of restricted growth in normal cells.

Furthermore, the regulation of proliferation in F9 cells during the change from a tumorigenic state to a parietal endodermal cell seems to be a multi-step event. Post-transcriptional regulation of *myc* affecting the half-life of primary transcripts is an early event in F9 differentiation. It precedes morphological differentiation and changes in cell cycle known to occur approximately 72 h after induction²⁵. Our studies using cycloheximide

as an inhibitor of protein synthesis indicate that synthesis of a short-lived protein, probably an RNase, is responsible for the relatively short half-life of *myc* RNA in undifferentiated F9 cells but also for the extremely short half-life of the differentiated cell. Whether this is the result of increased synthesis of one putative RNase or due to the synthesis of a different RNase remains to be determined. A protein controlling the level of stable *myc* RNA in 3T3 fibroblasts was suggested previously by Leder *et al.*¹². In contrast to *myc* RNA, the down-regulation of *p53* RNA occurs much later during the differentiation process. This reduction of stable *p53* RNA coincides with an alteration of morphology and changes in the cell cycle²⁵. One might speculate, therefore, that the post-transcriptionally regulated reduction of *p53* RNA—in contrast to the *myc* down-regulation—is a secondary event that can result from cell cycle changes.

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Note added in proof: Jeanteur and colleagues have shown that the reduced level of *c-myc* RNA in Friend cells induced to differentiate by Me₂SO is also due to a post-transcriptional control mechanism³⁶.

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Morphological transformation *in vivo* of human uterine cervix with papillomavirus from condylomata acuminata

John W. Kreider*†, Mary K. Howett†, Sue Ann Wolfe*, Gerald L. Bartlett*†, Richard J. Zaino*, Thomas V. Sedlacek‡ & Rodrigue Mortel§

Departments of * Pathology, † Microbiology and § Obstetrics and Gynecology, Milton S. Hershey Medical Center, Hershey, Pennsylvania 17033, USA

‡ Department of Gynecologic Oncology, Pennsylvania Hospital, Philadelphia, Pennsylvania 19107, USA

Carcinoma of the human uterine cervix has been associated with several infectious agents¹⁻⁵ including papillomaviruses^{6,7}. Papillomavirus group-specific antigen (GSA)^{8,9} and viral particles¹⁰ have been demonstrated in human condylomata acuminata (CA) and flat warts of the uterine cervix. Cell alterations consisting of nuclear enlargement, hyperchromasia, irregularity, binucleation and cytoplasmic clearing (koilocytosis) are often interpreted as mild to moderate dysplasia¹¹. Present evidence that human papillomavirus (HPV) is responsible for the development of these lesions relies on the association of GSA⁷ and virus particles⁸ in the affected tissue, fulfilling the first two of Koch's postulates¹². Direct proof of an aetiological relationship, however, requires induction of the CA change in normal, human uterine cervix after exposure to papillomavirus. Infecting human subjects with HPV is ethically unacceptable and no satisfactory alternative systems have been defined. Also, human cell cultures do not support growth or transformation by HPV. Here we report the first demonstration of the morphological transformation of human tissues with a human papillomavirus under controlled, experimental conditions. 'Transformation' is used here in its literal sense to refer to a heritable morphological alteration in the appearance of the cells. The use of this term does not indicate that the changes described are neoplastic, but they are identical to the dysplastic changes found in biopsies of uterine cervical CA. Our results demonstrate the direct involvement of CA virus in dysplastic change of human cervical tissue and indicate that the experimental system described may be useful in elucidating the contribution of human papillomaviruses to the pathogenesis of human cervical cancer.

We have described previously the neoplastic transformation by Shope papillomavirus of normal rabbit skin grafts placed on athymic mice¹³; this provided an environment suitable for the direct neoplastic transformation of xenogeneic tissues by papillomavirus. We used this system to test the hypothesis that human CA extracts can alter the morphology of normal human cervix to that associated with papillomavirus infections¹¹.

Athymic mice (*nu/nu* on a BALB/c background; Harlan Sprague-Dawley, Inc., Madison, Wisconsin) were housed in isolators supplied with sterile air, water and chow. Cervical tissue was obtained from four patients who underwent hysterectomy. Grafts (2×2×0.5 mm) were cut from the squamo-

columnar junction, and were held in medium with gentamicin. They were then rinsed and incubated for 1 h at 37°C in a phosphate-buffered saline (PBS) extract of human CA, with inactivated herpes simplex type 2 virus (HSV; 1×10⁷ plaque-forming units (PFU) ml⁻¹, irradiated for 12 min with 46 erg mm⁻² s⁻¹), with both viruses, or with PBS alone. The CA (total 20 g from 15 female patients) were minced and disrupted in 50 ml of PBS at 4°C with a Virtis homogenizer at 25,000 r.p.m. for 30 min. Supernatants and pellets were separated by sedimentation at 11,000g. HSV was produced, stored and titrated as described previously¹⁴.

The fragments of cervical tissue were then grafted beneath the renal capsules of the athymic mice. The kidneys were delivered through dorsal incisions and the renal capsule was nicked, and a cervical graft placed beneath it. The mice were placed on supplemented (0.01 mg ml⁻¹ trimethoprim and 0.05 mg ml⁻¹ sulfamethoxazole) drinking water for the duration of the experiment, after which they were killed, the kidneys extracted and fixed in formalin, and haematoxylin-eosin sections obtained. The presence of GSA was demonstrated with commercial reagents (Dakopatts, Accurate Chemical & Scientific Corp., Westbury, New York)^{8,9}.

About one-half of the cervical grafts persisted and formed cysts beneath the renal capsule. Grafts which were untreated or exposed to HSV alone were identical in appearance and comprised cysts (>1 mm in diameter), lined by a thin stratified squamous epithelium (Fig. 1a). In contrast, most of the grafts which had been infected with the CA virus were much larger and were lined with an epithelium often about 20-30 cells thick. The typical features of CA, including koilocytosis and accompanying nuclear hyperchromasia and binucleation, were observed (Fig. 1b,c). The morphological transformation described here was identical to that usually seen in cervical CA biopsies^{8,9,11}. Table 1 shows that the frequency of CA transformation in grafts treated with CA extract (7/22) was significantly greater ($P < 0.025$, Fisher's exact probability test) than in grafts treated with PBS alone (0/13). Similarly, treatment with CA extract and HSV increased ($P < 0.006$) the incidence of transformation (8/14) compared with grafts treated with HSV alone (0/9). The difference between the incidence of CA transformation in pooled data of all grafts treated with CA extract (15/36) and that in comparable pooled data of grafts not exposed to CA extract (0/22) is highly significant ($P < 0.0002$).

Strong and specific GSA reactions were found in the nuclei of the cells of the CA epithelium (Fig. 1c). Positive nuclei were focally distributed only in cells of the maturing and parakeratotic epithelial layers^{8,9}. GSA was detected in transformed grafts from three of the four patients studied and was not found in controls that had not been exposed to CA extract.

'Dot-blot' hybridizations using labelled HPV DNA probes were done on both the original CA supernatant and pellet which

Table 1 Morphological transformation by condylomata acuminata virus of human uterine cervical grafts transplanted beneath the renal capsule of athymic mice

Patient	Cervical pathology	Duration of grafts in mice (days)	Treatment group			
			None	CA	HSV	CA+ HSV
A	None	86	0/2*	3/6	—	—
B	None	70	0/4	1/5	—	—
C	Chronic inflammation	62	0/3	3/3	0/5	4/4
D	Chronic inflammation	60	0/4	0/8	0/4	4/10
Totals			0/13	7/22	0/9	8/14

CA, condylomata acuminata extract; HSV, herpes simplex type 2 virus suspension.

* Number of grafts with morphological transformation/total number of grafts.

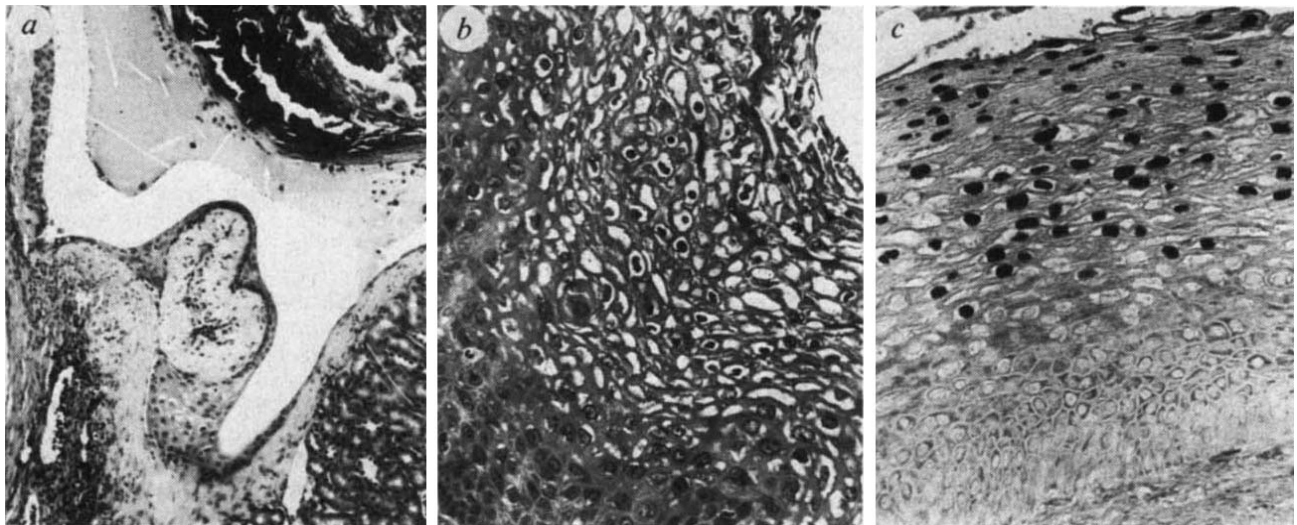


Fig. 1 *a*, Section of a cyst which developed from a control, untreated graft of human cervix, examined 2 months after placement beneath the renal capsule of an athymic mouse. Stratified squamous epithelium lines a cystic space. Haematoxylin-eosin stain. $\times 113$. *b*, Section of papillomatous epithelium which developed 2 months after grafting of cervical epithelium infected with CA extract. The epithelium is highly hyperplastic compared with the control (*a*) and there are extensive koilocytotic changes consisting of nuclear wrinkling, binucleation and perinuclear halos. Haematoxylin-eosin stain. $\times 245$. *c*, Section of papillomatous epithelium from a CA virus-infected graft which has been stained using the immunoperoxidase technique to demonstrate the presence of papillomavirus antigen. Maturing epithelial layers contain black nuclei which are strongly reactive with the antibody. The immature cells in the lower layers do not contain antigen. No counterstain. $\times 216$.

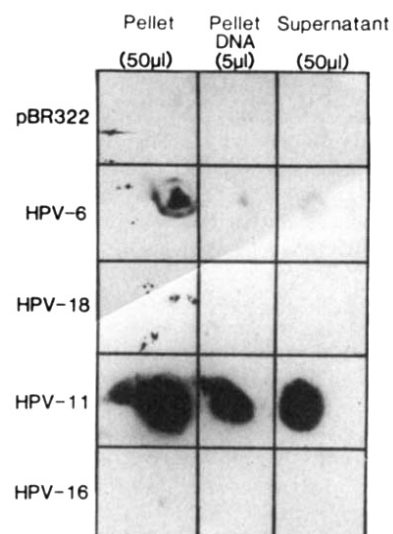
were the source of infectious virus (Fig. 2), and on DNAs extracted from the cervical grafts retrieved from the athymic mice (Fig. 3). CA supernatant, pellet, and DNA derived from the pellet, strongly bound the HPV-11 probe (Fig. 2); less intense binding was observed with the HPV-6 probe. Probes containing HPV-16, HPV-18 or pBR322 vector alone did not bind. DNA samples from four uninfected cervical grafts and from four cervical grafts (same human donor) experimentally infected with the CA extract were spotted onto nitrocellulose paper in varying amounts and hybridized with the labelled probes. The HPV-11 probe (Fig. 3) hybridized strongly to DNA from grafts infected with CA extract; HPV-6 DNA hybridized to a lesser extent, and HPV-16 and -18 DNAs showed no hybridization. Similar probe binding was seen in the other three CA graft samples, but not in the normal, uninfected cervical grafts.

The results presented here demonstrate that koilocytotic transformation of human cervical tissue may be induced with papil-

lomavirus extracted from human CA. The presumption of an aetiological relationship between the two has, until now, depended on the association of human wart virus markers with the cervical lesions. Our study fulfils the third of Koch's postulates¹² in supporting the causal relationship. It is extremely unlikely that an agent other than a papillomavirus could be responsible for our results as the morphological changes, and presence of GSA and HPV DNA, are diagnostic of papillomavirus infections. The use of labelled HPV-plasmid DNA probes demonstrated hybridization of both HPV-11 and -6 DNAs to the CA extract and to the infected cervical tissue. Both HPV-6 and -11 are found in naturally occurring vulvar CA, although HPV-6 is more prevalent¹⁵. As HPV-11 was predominant in our CA extract, it is possible that one or a few CA in our pool were rich in HPV-11. HPV-6 and -11 are also 25% homologous to each other¹⁵, so the hybridization reaction with HPV-6 DNA could represent cross-hybridization to HPV-11 or

Fig. 2 HPV DNA content of the naturally occurring human vulvar condyloma tissue pellet and supernatant. Dot-blot hybridization was done using ³²P-nick-translated probes consisting of DNA from HPV-6, -11, -16 or -18 in a plasmid vector. Tissue pellet (50 μ l of a 1:1 suspension in buffer), DNA extracts of tissue pellet (5 μ l), or low-speed supernatant (50 μ l) were spotted onto nitrocellulose. All probes were labelled to approximately the same specific activity. Filters were exposed to X-ray film for identical times. Vector (pBR322 without HPV DNA) was used as a control probe.

Methods. Cloned DNAs of HPV-6, -11, -16 and -18 were provided by Drs P. Howley (Bethesda, Maryland) and H. zur Hausen (Freiburg, FRG). *Escherichia coli* (strain HB101)¹⁸ recombinant DNA constructs were grown in bulk and plasmids amplified with chloramphenicol. Plasmid DNA was extracted with lysozyme and Triton X-100 and purified on CsCl/ethidium bromide equilibrium gradients. Plasmid identities were verified by restriction endonuclease cleavage and agarose gel electrophoresis. Plasmids were ³²P-nick-translated to 1×10^8 c.p.m. per μ g DNA (Amersham, Arlington Heights, Illinois). DNA of high relative molecular mass was obtained by phenol and phenol/chloroform extraction of the pellet of the homogenized CA pool used for preparation of the CA extract. After ethanol precipitation, re-solubilized DNA samples were bound to nitrocellulose filters and hybridized under stringent conditions (35.6% formamide, 10% dextran sulphate, 0.845 M Na⁺, 46°C) with the ³²P-nick-translated HPV DNA probes. The filters were washed, dried, and autoradiographed at -70°C.



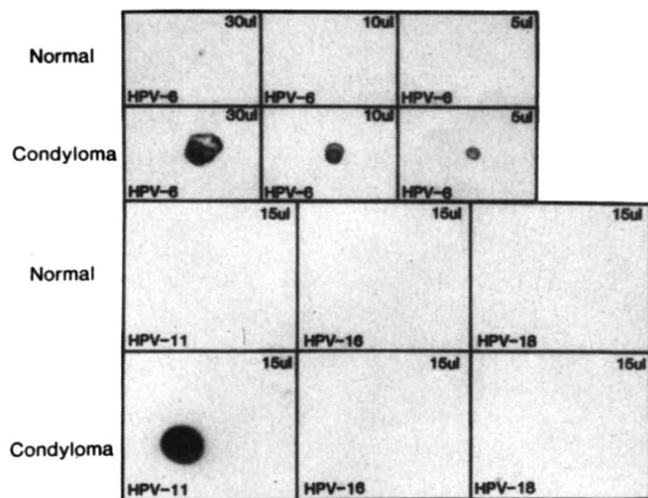


Fig. 3 HPV DNA content of normal and CA virus-infected human cervical grafts. Dot-blot hybridization was done using ^{32}P -nick-translated probes consisting of DNA from HPV-6, -11, -16 or -18 in a plasmid vector. DNAs were spotted onto nitrocellulose in volumes of 30, 15, 10 or 5 μl . Same probes and hybridization conditions as in Fig. 2.

a true minority population of HPV-6. The high reactivity of HPV-11 in the extract was reflected in the HPV content of the experimentally infected grafts. HPV-11 is commonly encountered in both cervical CA and laryngeal papillomas¹⁵. In contrast, HPV-16 and -18 have been detected in cervical cancer^{16,17}, but are found infrequently in vulvar CA. Our results strongly support the conclusion that the CA change which we have induced in grafts of human cervical tissue was induced by HPV-11.

Ultraviolet irradiation of HSV under the conditions which we used destroys the cytolitic properties of the virus without substantially reducing its ability to transform hamster cells *in vitro*¹⁴. Ultraviolet light-inactivated HSV alone did not alter the appearance of the cervical transplants nor did it affect the appearance or behaviour of CA virus-transformed tissue; this does not exclude the possibility of participation of herpes virus in the development of human cervical cancer nor does it preclude subsequent effects in our system, since the observation period was brief.

Our report demonstrates a unique method which may be useful in the analysis of transformation mechanisms for the other human papillomaviruses. The principles of this approach can easily be extended to other tissues or other oncogenic agents which are thought to be involved in the induction of human neoplasia. More specifically, this system offers an unparalleled opportunity to evaluate the relative contribution of human papillomaviruses and other possible co-factors to the induction of human cervical cancer.

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Response regulation and sensory control in *Halobacterium halobium* based on an oscillator

Angelika Schimz & Eilo Hildebrand

Institut für Neurobiologie, Kernforschungsanlage Jülich, D-5170 Jülich, FRG

Swimming bacteria reveal a simple mode of behaviour: their flagella rotate clockwise or counterclockwise by turns and they respond to external stimuli by changing the duration of reversal intervals, thereby migrating in gradients towards attractants or away from repellents in a 'biased three-dimensional random walk'. The molecular mechanism that regulates the directional change of flagellar rotation is unknown. The light-controlled behaviour of the archaeobacterium *Halobacterium halobium* bears many similarities to chemotaxis in eubacteria¹, and offers the advantage of studying the events with a better resolution in time. We have now found in *H. halobium* that the distribution of the interval length between reversals is log normal and not exponential, as it is in *Escherichia coli*. During the interval, the responsiveness of the cells to attractant stimuli changes in a sawtooth-shaped manner. These results indicate that the intervals between reversals in this organism are regulated by an oscillator which is influenced in opposite directions by attractant and repellent stimuli.

Halobacteria are polarly flagellated and swim in either direction of their long axis. About every 10-20 s, depending on the bacterial strain, the cells spontaneously reverse their swimming direction as a result of change of flagellar rotation from clockwise (CW) to counterclockwise (CCW) or vice versa². Light stimuli influence this behaviour through two retina-dependent sensory photosystems³⁻⁵ which, according to their maximal spectral sensitivity, are called PS 370 and PS 565 (ref. 6).

Single bacteria in a suspension from the stationary growth phase were observed through a microscope connected to a video system. Their movements were followed by eye, and the intervals between reversals of the swimming direction were measured with an electronic stop-watch. Comparison of even- and odd-numbered intervals (*t*-test) indicated that subsequent intervals between spontaneous reversals measured in an individual cell did not differ significantly, that is, the bacteria have no preferred swimming direction. Variations of spontaneous interval lengths measured with single cells were similar to those from a population of cells, the standard deviations covering a range of 25-59% in both cases. Differences between individual cells, however, contributed slightly to the overall variation (analysis of variance, $P = 0.025$). The shapes of the frequency distributions of spontaneous reversal intervals obtained from single cells and from a population of cells were also similar (Fig. 1).

An increase of yellow-green light or a decrease of ultraviolet light prolonged the interval to the next reversal and was therefore regarded as an attractant stimulus. An increase of ultraviolet light or a decrease of yellow-green light, on the other hand, shortened the interval to the next reversal and was considered to be a repellent stimulus⁶⁻⁸ (Fig. 1). We found that a stimulus changed one reversal interval only; the following interval was equal to the pre-stimulus (spontaneous) one. Also the responsiveness to a stimulus was the same whether it was applied after a spontaneous reversal or after a stimulus-determined one (Table 1). Thus, the cells quickly adapt to constant light conditions.

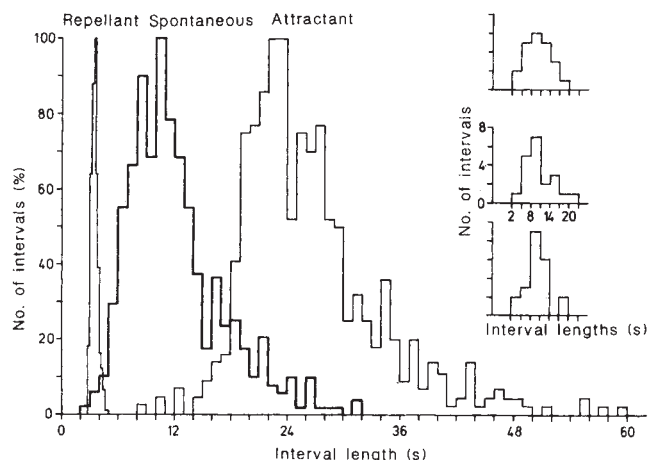


Fig. 1 Frequency distributions of interval lengths between spontaneous reversals (mean $\pm$ s.d. = 12.27 ± 5.13 s, 443 intervals) and intervals obtained after a repellent stimulus (3.51 ± 0.36 s, 491 intervals) or an attractant stimulus (26.59 ± 7.47 , 525 intervals). Each histogram contains data from one culture (*H. halobium*, strain ws) only. Frequencies were normalized with respect to the maxima. Repellent stimulus: step-like light increase of 6×10^{13} photons $\text{mm}^{-2} \text{s}^{-1}$ at 370 nm. Attractant stimulus: step-like light decrease of 6×10^{13} photons $\text{mm}^{-2} \text{s}^{-1}$, 370 nm. Stimuli were applied 2 s after a spontaneous reversal had been observed. The interval length between the reversal before and after the stimulus was measured. Background light was white light, $250 \mu\text{W mm}^{-2}$. For technical details see ref. 1. Insert: distribution of spontaneous intervals from 3 single cells, 20 events each.

Table 1 Behaviour after a spontaneous or stimulus-determined reversal

1st interval		2nd interval	
Stimulus	Interval length (s)	Stimulus	Interval length (s)
None	8.8 ± 0.7	None	8.7 ± 0.8
Attractant	17.4 ± 0.6	None	8.7 ± 0.7
Repellent	5.3 ± 0.4	None	8.7 ± 0.8
None	8.4 ± 0.4	Attractant	17.9 ± 0.9
Attractant	17.7 ± 1.2	Attractant	18.5 ± 1.1
Repellent	5.5 ± 0.2	Attractant	17.9 ± 1.1
None	8.7 ± 1.2	Repellent	5.1 ± 0.3
Attractant	17.1 ± 1.3	Repellent	5.4 ± 0.3
Repellent	5.2 ± 0.4	Repellent	5.3 ± 0.3

Two successive reversal intervals were measured in an individual cell. Attractant stimuli (light decrease of 6×10^{12} photons $\text{mm}^{-2} \text{s}^{-1}$ at 370 nm) or repellent stimuli (light increase at 370 nm, same intensity change) were applied during the first and/or second interval, 2 s after a reversal. Each value is the mean $\pm$ s.e.m. of 30 measurements.

The shape of the frequency histograms (Fig. 1) became symmetrical when the logarithm of interval lengths was taken, and the distributions of log interval lengths were normal. Also, no significant difference in the type of distribution was found between spontaneous and stimulus-determined intervals as revealed by χ^2 -test. From the exponential distributions of interval lengths in *E. coli*, Berg and co-workers^{9,10} concluded that reversal induction was a poissonian process. In their two-state model, spontaneous transitions between CW and CCW rotation occur with a constant probability per unit of time. The distribution of intervals found in *H. halobium*, on the other hand, led us to assume that the interval length in this organism is regulated.

To obtain more information about the regulation of the response, we stimulated bacteria at different times after a reversal. The effectiveness of a light stimulus in biasing the probability of the time of the next reversal was not constant during an interval but depended strongly on the moment at which the stimulus was delivered. The effectiveness of an attractant stimulus, represented by the length of the reversal interval, first increased continuously to reach a maximum at a delay of

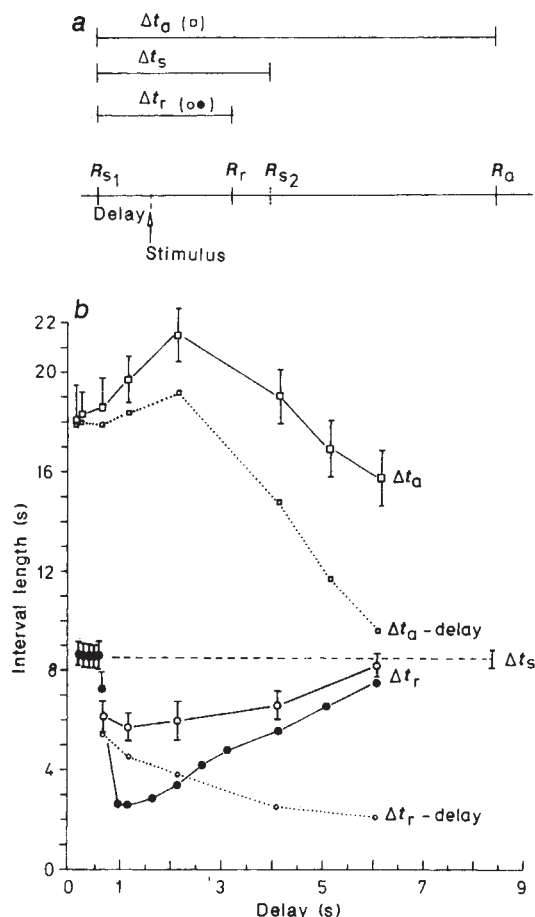


Fig. 2 Effectiveness of attractant and repellent stimuli in the course of time between two reversals of the swimming direction. **a**, Experimental programme. Cells were stimulated at given delays after a spontaneous reversal. The shortest delay was 200 ms, due to the experimenter's reaction time. Δt_s is the interval length between two spontaneous reversals, R_{s1} and R_{s2} . Δt_d is the time between R_{s1} and R_d , the reversal determined by an attractant stimulus. Δt_r is the time between R_{s1} and R_r , the reversal on a repellent stimulus. **b**, Upper curve, the effect of attractant stimuli; lower curves, the effect of repellent stimuli. Open symbols, light intensity changes of 6×10^{12} photons $\text{mm}^{-2} \text{s}^{-1}$ at 370 nm; filled circles, 10-fold intensity. The dotted lines show the interval length Δt minus delay. Background, white light, $250 \mu\text{W mm}^{-2}$. Temperature, 22–24 °C. Each point is the mean $\pm$ s.e.m. of 30 measurements. At a delay of 6 s, less than 10% of the cells reversed before the stimulus was delivered and were therefore discarded. Similar curves were obtained when the cells were stimulated through PS 565.

about 2 s and then became weaker again (Fig. 2b, upper curve). Subtraction of the delay shows that the initial increase of the curve is not a result of the increasing delay (dotted curve). Measurements of bacterial strains with different spontaneous reversal intervals up to 24 s showed that attractant stimuli always exert their maximal effect at a delay of $\sim 1/5$ th of the spontaneous interval length (not shown).

When repellent stimuli were applied shortly after a spontaneous reversal, the cells did not respond at all¹¹; even a 10-fold stimulus did not shorten the reversal interval (Fig. 2b, lower part). This period of 'absolute refractoriness' lasted ~ 500 ms and was followed by a period during which the reversal interval gradually became shorter. With further delay, the interval length increased again and finally approached the pre-stimulus value. In the case of repellent stimuli, the latter effect is, of course, a consequence of the increasing delay. The response latency (Δt_r minus delay), which can be taken as a measure of responsiveness to repellent stimuli, becomes shorter with increasing delay. The duration of absolute refractoriness did not depend on the length of the spontaneous interval; it also lasted for ~ 500 ms in cells

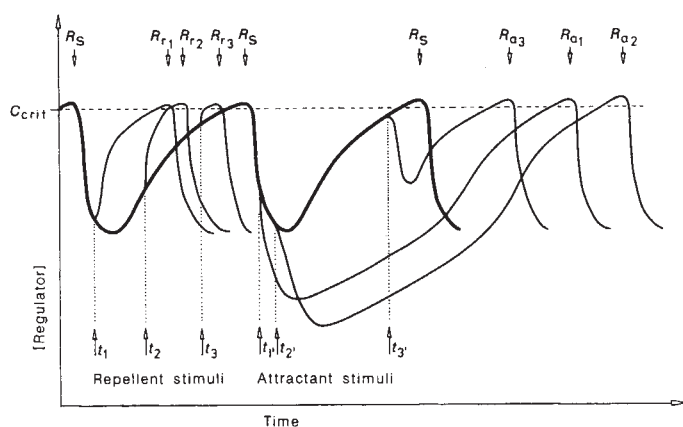


Fig. 3 Schematic drawing to explain response regulation on the basis of an oscillator. The heavy line, which reflects the time-dependent change in responsiveness to attractant stimuli (see Fig. 2), represents the shape of self-sustained oscillation and is regarded as the 'regulator' concentration in the absence of stimuli. Light lines illustrate the hypothetical change of regulator level after repellent or attractant stimuli applied at different times, $t_1 - t_3$ or $t_1' - t_3'$, during an interval. R_s , Spontaneous reversals; R_r , reversals after a repellent; R_a , reversals after an attractant stimulus. Dashed line, critical concentration (C_{crit}) at which a reversal is induced. For further explanations see text.

which spontaneously reversed ~every 20 s. Similar curves to those seen in Fig. 2 were obtained when the stimulus was given after a stimulus-determined reversal instead of a spontaneous one (not shown).

The interval distributions shown in Fig. 1, together with the sawtooth shape of the attractant curve, indicates that an oscillator controls the behavioural pattern in *H. halobium*. Figure 3 shows our present hypothesis on the regulation of reversal intervals and sensory control in *H. halobium*. We assume that the time-dependent change in the responsiveness to attractant stimuli (Fig. 2) reflects the time-dependent change in the concentration of a regulatory substance X. This substance, which is thought to have a similar function to the 'response regulator' proposed by Koshland¹² for eubacteria, oscillates within a certain concentration range and triggers a switch to change flagellar rotation from CW to CCW or vice versa when a critical level is reached. Sensory input signals should transiently influence the level of the regulatory substance and thereby shift the phase of the oscillator. The result is one altered interval, according to the observations shown in Table 1, that is, only one period of oscillation is disturbed. As attractant and repellent stimuli have opposite effects on the interval length, we assume that they alter the concentration of X in opposite directions.

Our model shows how the effectiveness of an attractant stimulus is greatest when the concentration of X is farthest from the critical value. It also explains how the response latency for a repellent stimulus (Δt , minus delay) in Fig. 2b progressively decreases while X rises towards the critical value. The shortest latency that we could measure between stimulus and first observable movement of the cell in the new direction was 1.5 s, perhaps because of time taken following the oscillator. A sawtooth shape for the repellent curve cannot be expected because of the refractory period. Refractoriness may be an inherent property of the oscillator or should at least be closely linked to it or to adjacent steps in the signal-processing pathway. This is supported by the fact that simultaneously applied attractant and repellent stimuli cancel each other even during the refractory period (not shown), indicating that repellent stimuli are recognized during this period and that cellular signals are generated and integrated. We assume that the output of this integration link influences the concentration of the regulatory substance X.

Our results show that response regulation in the archaeobacterium *H. halobium* is fundamentally different from that in *E. coli* or *Salmonella typhimurium*. Note that *H. halobium* modu-

lates reversals of flagellar rotation from CW to CCW or vice versa, while *E. coli* controls the direction of flagellar rotation and swims only when the flagella rotate CCW.

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Porin channel triplets merge into single outlets in *Escherichia coli* outer membranes

A. Engel*, A. Massalski*, H. Schindler*, D. L. Dorset† & J. P. Rosenbusch*‡

* Biozentrum, University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland

† Medical Foundation of Buffalo, 73 High Street, Buffalo, New York 14243, USA

‡ European Molecular Biology Laboratory, PO Box 10.2209, D-6900 Heidelberg, FRG

Previous observations on the structural¹⁻⁴ and functional⁵ properties of porin, the matrix protein of *Escherichia coli*, have indicated that the channel-forming trimers span the outer membranes of the bacterial cell, forming a molecular sieve⁶. By using electron microscopy and image reconstruction, we demonstrate here that three channels on the outer surface of the cell merge into a single channel at the periplasmic face. Conductance measurements using conditions under which single activated triplets could be observed led us to conclude that the three individual consecutive closing steps reflect three channels within a single trimeric unit. Statistical analysis of conductance levels revealed that the first relaxation step is distinctly smaller than the two subsequent channel closings. This functional observation can be explained if the channels of porin trimers coalesce.

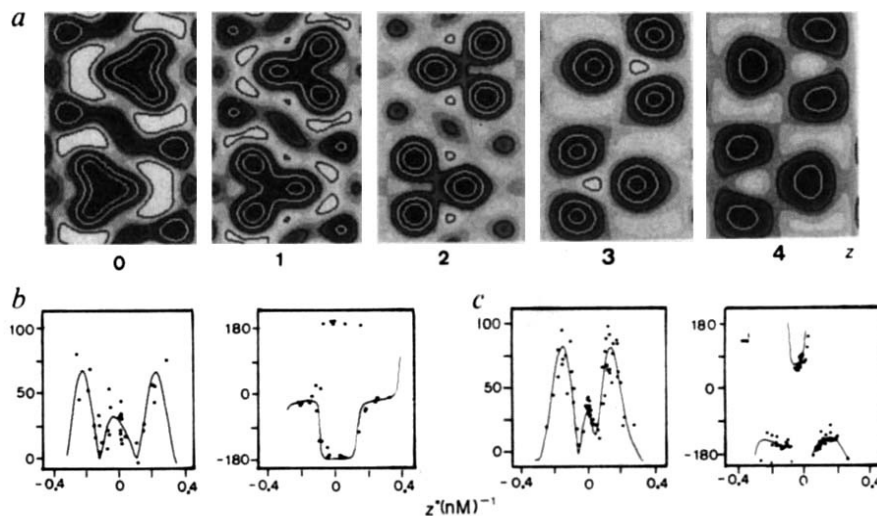
Porin contains a large fraction of polar amino-acid residues¹ and does not exhibit a single long hydrophobic stretch⁷. It is arranged predominantly in an anti-parallel β -pleated sheet structure⁸. Though different from hydrophobic membrane proteins such as bacteriorhodopsin, a transmembrane arrangement of porin may be envisaged by postulating extensive hydrogen-bonding networks among polar residues⁹. Such unusual properties prompted further investigation of its function and structure.

We used membrane vesicles reconstituted from homogeneous porin trimers and phospholipids at high² or low¹⁰ protein-to-lipid ratios, all other conditions being identical. High protein concentration (final protein-to-lipid weight ratios of 6) yielded two crystal forms, one rectangular (unit cell dimensions $a = 13.7$ nm, $b = 7.9$ nm; see Fig. 1) and the other hexagonal (lattice constant $a = 7.9$ nm). Projections of negatively stained membranes were recorded over tilt angles of $\pm 60^\circ$ for both rectangular and hexagonal crystals and were used for three-dimensional reconstructions as described elsewhere^{11,12}. Fourier terms of 16 rectangular and 11 hexagonal lattice lines were measured. They



Fig. 1 Porin membranes obtained by reconstitution with dimyristoyl phosphatidylcholine. After freeze-drying and tungsten shadowing a regular surface modulation becomes visible. Membranes are frequently broken along lattice lines. From shadow lengths and direction (arrow), and a shadowing angle of 30° , the thickness of a single layer is estimated to be 5.5 nm. Scale bar, 50 nm.

Fig. 2 Three-dimensional reconstruction of two-dimensional porin crystal lattices from projections recorded over tilt angles of $\pm 60^\circ$. *a*, Serial sections across a three-dimensional map of the rectangular crystal form sliced parallel to the membrane plane. The point of reference of the calculated sections is near the inner surface of the membrane (at the left). Individual panels represent sections sampling the membrane at 1-nm intervals. Contours divide the density range of the three-dimensional map into five equal levels. The figure illustrates the divergence of a single channel to a triplet channel (from left to right). *b*, Amplitude (left) and phase diagram (right) of the prominent 2,1 lattice line from the rectangular lattice. A distinct symmetry with respect to the membrane plane is evident. The overall phase residual was 17° or, if symmetry along the z^* axis was enforced, 16° . *c*, Amplitude and phase diagram of the 2,1 lattice line from the small hexagonal lattice. The overall phase residual in this instance was 12° , and 15° if symmetry along the z^* axis was enforced.



extend to a resolution of 2.3 nm within the plane of the membrane and 2.8 nm perpendicular to it (Fig. 2). The smooth amplitude and phase curves, interpolated by a $\sin(x)/x$ function, show a distinct symmetry with respect to the membrane plane (Fig. 2*b, c*), which is due to a (crystallographic) stacking of two porin layers with trimers positioned face-to-face¹². Face-to-face dimers of porin trimers also appear to constitute the basic building block in porin crystallization to large three-dimensional crystals³. Contour maps of the two different crystal forms clearly show the transmembrane channels (Fig. 3). Sectors of single membrane layers each contain two trimers corresponding to a protein relative molecular mass of 220,000 (220K) and lipids of 35K. This 6:1 protein-to-lipid weight ratio appears to be the same in the small hexagonal and the rectangular lattices.

The triplet stain indentations, which were previously tentatively designated as pore orifices¹³, can now be seen as continuous, stain-penetrated channels (Figs. 2, 3). Their structures are strikingly similar in both polymorphic crystal variants. The triplet channels on one side of the membrane merge into a single channel on the opposite membrane surface (Fig. 2*a*). In agreement with earlier results obtained from freeze-dried and shadowed specimens¹³, we conclude that three orifices are situated on the cell surface, implying that the single outlet is located

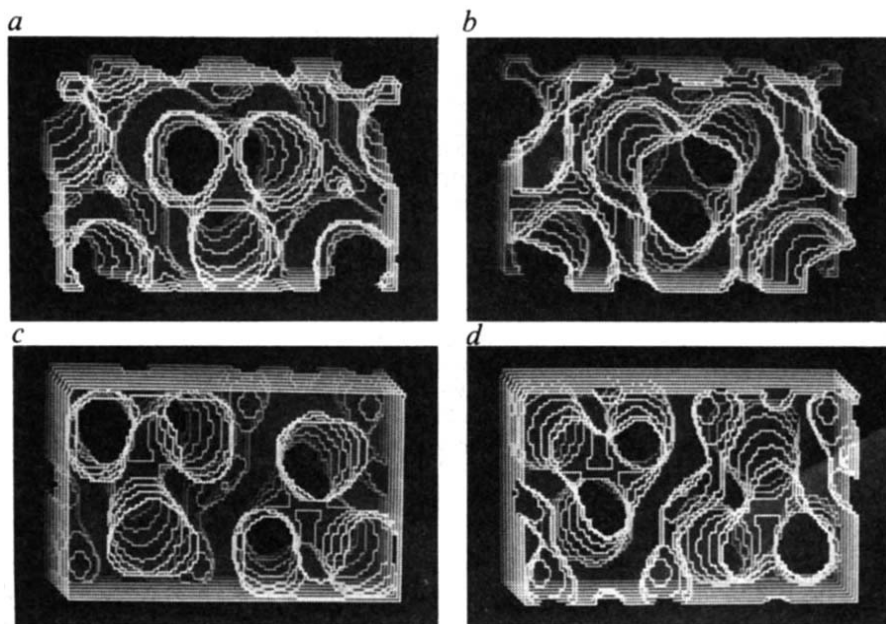


Fig. 3 Three-dimensional reconstruction of porin membranes. *a, b*, The small hexagonal crystal form. *a*, A view from the outer surfaces towards the inside; *b*, the opposite view. *c, d*, The two perspectives of the rectangular lattice in analogous sequence. In both crystal habits, two trimers are shown within an area of 13.7×7.9 nm. In the hexagonal form, a central triplet channel is surrounded by four quarters of neighbouring trimers. In the rectangular form, the two triplets contained within the area shown are rotated by 180° around an axis perpendicular to the membrane plane (see Fig. 2*a*). Contours along the z -axis are 0.5 nm apart, with increasing brightness from back to front. The protein (and lipid) mass with a density of 1.2 K nm^{-3} , is shown as a grey body.

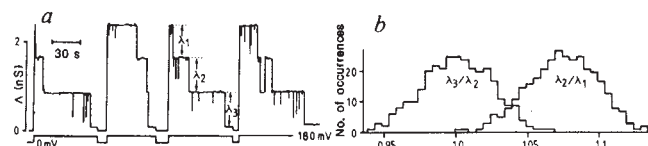


Fig. 4 Conductance levels of channel triplets. Single-step relaxation was observed consistently, with occasional rapid fluctuations between open and closed states. **a**, Four consecutive closures of a channel triplet. Switching the voltage on and off (180 mV; line at the bottom of **a**) yields a representative series of voltage-dependent opening and closing events of one channel triplet. Planar membranes were formed from soybean phospholipid and homogeneous porin trimers with undissociated glycolipids (lipopolysaccharides) at a protein-to-lipid weight ratio of 6×10^{-8} . The average number of trimers per bilayer was 30 in all experiments. The voltage was slowly increased until a single trimer was activated; this occurred at 180 mV in the trace shown. About 12 consecutive opening-closing cycles were observed with the first activated trimers before activation of multiple-channel triplets was recorded¹⁰. Solutions in the two-compartment cell were buffered with 20 mM HEPES, pH 7.0, and contained 1 M NaCl. **b**, Conductance steps (λ) represent the closing of individual channels within a porin trimer. They are slightly but distinctly different. Differences were analysed from recordings of 326 opening and closing cycles using 27 independently activated channel triplets (of which that in **a** is one). In terms of conductance ratios (λ_i/λ_j , where subscripts 1–3 indicate conductances of the first, second and third closure step, respectively), the first step was 7% smaller than the second and third.

on the periplasmic side. Attempts to confirm the orientation of these orifices by surface reconstruction of freeze-dried, shadowed cell envelopes are now in progress¹⁴.

In view of the observed channel morphology, a re-investigation of previous functional findings^{5,10} appeared necessary. Reconstitutions were performed with protein-to-lipid ratios eight orders of magnitude smaller, allowing individual steps of channel closure to be observed within single triplets. Figure 4a shows a typical trace of repeated openings of a single channel triplet, and three consecutive closing events corresponding to individual channels within one triplet. Statistical analysis (Fig. 4b) shows that the peak of the conductance of the first step is significantly smaller than that of the successive second and third steps, although the difference determined amounts to a mere 7%. A qualitative explanation of this phenomenon would imply that the single orifice on one side constitutes the limiting area for ion flux if all three channels are open. Once one of the channels is closed, the area of individual channels becomes limiting. This can be inferred from the geometry of coalescent triplet channels (Fig. 3), in which the area of the common orifice is smaller than the sum of the areas of individual channels.

The molecular mechanism of channel opening and closing, however, remains to be understood. Relatively small conformational changes of subunits may occur, with effects comparable to those of the allosteric changes seen in haemoglobin on its water-filled pore¹⁵, although more drastic changes might be expected to occur in porin. Rotation and tilting of subunits, analogous to the conformational changes suggested to cause the opening and closing of gap junction channels¹⁶, are also conceivable. In any event, the correlation of functional properties with structural alterations seems possible. Attempts to visualize the open and closed states of the channels in three-dimensional reconstructions of stained or unstained preparations are in progress. In conjunction with the high-resolution X-ray structure^{3,4} and an improved understanding of the internal organization of porin⁹, the configuration of its water-filled channels across the bacterial outer membrane should be elucidated soon.

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Physical and functional repetition in a bacterial ice nucleation gene

Robert L. Green & Gareth J. Warren

Advanced Genetic Sciences, Inc., 6701 San Pablo Avenue, Oakland, California 94608, USA

Nucleation of a physical process is distinct from catalysis, and as the function of a protein it is highly unusual. The ability to nucleate ice formation in supercooled water is a property of some members of the bacterial genera *Erwinia*, *Pseudomonas* and *Xanthomonas*^{1–3}. This property is implicated in the ability of bacteria to cause frost injury to plants. Orser *et al.*⁴ have demonstrated that a single small region of DNA encodes this phenotype, and there is evidence that phosphatidylinositol is involved⁵. Biological ice nuclei are also found in the haemolymph of certain freeze-tolerant insects⁶. It is thought that an ice nucleation site must bind water molecules in an orderly array resembling an ice crystal. We have now determined the sequence of an ice nucleation gene from *Pseudomonas syringae*. It predicts a translation product whose structure is consistent with the role of template, containing 122 imperfect repeats of the consensus octapeptide Ala-Gly-Tyr-Gly-Ser-Thr-Leu-Thr. All the repeats are contiguous and higher-order periodicities are superimposed on the octapeptide pattern throughout. Deletion analysis showed that most regions of the repeating structure are not essential for function. Up to 68 octapeptides could be deleted without abolishing ice nucleation, but all deletions showed reduced activity. This indicates that the repeated peptides contribute individually to the nucleation process, as expected if they act as individual units of a water-binding array.

We cloned sequences conferring ice nucleation activity from *P. syringae* strain S203 (J. Lindemann, unpublished). Ice nucleation-active (INA⁺) clones of *Escherichia coli* were detected among a library of *EcoRI/BamHI* fragments, and a 4.5-kilobase *EcoRI/BglII* fragment conferring the INA⁺ phenotype was subcloned into vector pUC8 (ref. 7). Its sequence was determined and is presented in Fig. 1. It contains a single long open reading frame (which we designate *inaZ*), whose end points are indicated in the sequence. Through most of this reading frame, but nowhere outside it, there is a striking degree of sequence reiteration, with a 24-base periodicity. The consensus repeat (although not perfectly conserved in any position) is GCCGTTATGGCAGCAGCTGACC. The greatest variability between repeats occurs at each codon's third base, suggesting that selection has operated on the translation product of this sequence. The sequence of the predicted translation product contains exactly 1,200 amino acids (Fig. 2a); its relative molecular mass would be 118,610. Exactly 122 contiguous repeats of the consensus octapeptide AGYGSTLT can be recognized in it (residues 176–1,151). Two higher orders of periodicity were detected by an algorithm which plots self-comparison

1 AGATCTGTCGCGCGGACGG ATCGATCAGCGTCTGGTGTCT GTATGTCGAGCACTACCTGA GACCGGAGTTGTTCCCGGCG ATTCTCGAACACGACCTGAA CGAATCGCTCAGTGAACCTCT
 121 ACGCCCGCGCTATGACATT CATTACGGTCGGGTGCGCTT CGACATGGTCCCGACGGCGC TGCACAGCGAAGCAGCGCT GCACTGAAGGTTTCACTGGG TAGCCCGGGCGCTGCGCATTTG
 241 CACGTGTCAATTATGATCGG AAGATCGGTTGATCGACTG TGACCTCGAATACTGGCGTC ATGATGCTATTATGTCGCG GCAGAGGTGAACGCGCAATG AATGCCGGTGTGGCAAAACC
 361 ACTTCCCAAGCGCGGTCTGT GCCACCGTTAGTTTCGCTGTT TTCTATAAATAAATACTCAT TTTGTTGATAAAGATGTGTG CAATGTACAGCGCTTTTAAT AGGTTTTGTGTAATGCATGC
 481 ATTTGATTAAACAAAAAAT TAATGAGCATTATTCTATTG TTGCTGGCGGGGTGGCGTTA AATTAGTTGGTTTTTAATG GAATTTCCAGAGTAATTGT TCTAGTTTTCAGCGGATTTA
 601 AGAAAAATCTTAATATTAAT TAATGTTTGTAAATGGGGTG TGGTAGGTTTTTGGTGTGGA AGGATAGGCGCTGTTTATGT GGTATTATTTCGACGATCGT TCCATGTTTGGATTAAGGCT
 721 GACATGGCAGTTGTCTATA AAGCAATGCTTGATAAGTGC GGTGCTTTTATTAAAGGA TCTATGAGGATGCTGTAATG AATCTCGACAAGCGTTGGT GCTGCGTACCTGTGCAATA
 841 ACATGGCCGATCACTGCGGC CTTATATGCGCCGCTCCGG CACGGTGAATCCAGATACT GGCAGTCAACCAAGCGCGCAT GAGAAATGGTCTGGTCGGTTT ACTGTGGGCGCTGGAACCA
 961 GCGCTTTTCTAAGCGTGCAT GCCGATGCTCGATGGATTGT CTGTGAAGTTGCGGTTGCG CACATCATCAGTCTGGAAGAG CCGGGAATGGTCAAGTTTCC GCGGGCCGAGGTGGTTTCATG
 1081 TCGCGACAGGATCAGCGCG TCACACTTCATTTCGGCAGC TCAGGCGCAGCTCGCTCAA CATCAACATCAACGTTAAGC CCAATGCCACTGCCATACC CACGCCCATCGCTGCCGTAG
 1201 CAAGTGTACGTTACCGGTG GCCGAACAGGCCGCTCATGA AGTGTTCGATGTCGCGTCCG TCAGCGCGGCTGCCGCCCA GTAAACACCTGCGCGTACG GACGCCGAGAAATGTGACAG
 1321 CGGCCACTTACGCGCAGCAG TTGAGTGGCGACAAATCAGC TCGTCTGATTGCCGTTATG GCAGTACGAGACGCTGGC AACCACAGTGAATCTAATTGC CGTTATGGAAATACAGGCA
 1441 CCGCGGCTCCGACAGCTGG CTGGTCTGCTGGCTATGGAAG CACCCAGCAGCGCGGTGGG ACAGCGCGCTGACAGCGGGT TACGGCAGCAGCCAGACCGC CCGCGAAGGCAGCAACCTGA
 1561 CCGCAGGGTACGCGCAGCAC GGCACGCGCAGGCTCGGACAG TTCGCTGATCGCGGTTACG GCAGTACTCAGACTTCGGGC GGGGACAGCTCACTACAGC GGGTTACGCGCAGCAGCAAA
 1681 CCGCTCAGGAAGCGACCAAT CTCACCGCTGGGTATGGCAG CACCGGCACGCGAGGCTCGG ACAGCTCCTTGATCGCGGCT TATGGCAGTACACAAACCTC GGGAGGCGACAGTTCGCTGA
 1801 CCGCGGGCTACGCGAGTACG CAGACGCGCCAGGAGGGCAG CAATCTGACGCGGGGTACG GCAGCAGGGTACAGCAGGT GTCGACAGCTCTCTGATCGC GGGATACGCGCAGCAGCAGA
 1921 CCTCGGAAGTGACAGCGCC CTGACCGCAGGCTATGGCAG CACGCAAAACGCGCCAGGAAG GCAGCAATCTCACTGCTGGG TATGGCAGCAGCGGACGCG AGGTTCCGACAGCTCGCTGA
 2041 TCGCGGTTACGCGCAGCAG CAAACCTCGGCGAGTGACAG CTCGCTCAGCGCGGGTACG GCAGTACGACAGCGGCTCAG GAAGGCGCATTCTGACGGC GGGTACGCGCAGCAGGGTA
 2161 CAGCAGGTGTCGACAGTTTC TTGATCGCGGATATGGCAG CACGCAGACCTCGGGAAGTG ACAGTGCCTGACAGCGGGT TACGGCAGCAGCAAAACGGC CCAGGAAGGCGCAGCAACCTGA
 2281 CCGCGGGCTACGCGCAGCACT GGCACGCGAGGTGCCGACAG TTCGTTGATCGCGGATATG GCAGCAGCAGCAGCTCAGGC AGCGAAAGTTCGTTACCGC AGGCTATGGCAGTACCCAGA
 2401 CTGCGGCTGAGGGCAGCACC CTGACGCGCGGATATGGCAG TACCGGAACAGCTGGCGCTG ACAGCTCGCTGATCGCGGCT TACGGCAGCAGCAAAACCTC GGGCAGTGAAGCTCGCTCA
 2521 CCGCAGGTATGCGCAGTACC CAGACCGCAGCAGCGGCGAG CGTACTCACATCAGGCTATG GCAGTACGCAAAACGCGCGG GCTGCCAGTAACCTCACCAC CGGTTACGGAAGTACAGGTA
 2641 CCGCAGGTACGAGAGTTTC ATCATTGCGGGTTATGGAAG TACACAGACAGCGGGCCACA AAAGTATCCTGACCGCTGGT TATGGCAGTACTCAGACGGC CAGGGACGTTAGCGACCTGA
 2761 TTGCGGGCTATGCGCAGTACG GGAACCGCAGGCTCGGGCAG TTCGCTGATCGCAGGTTATG GCAGCAGCCAGACCGCGAGT TACAGAAGCATGCTGACCGC CGGTTATGGCAGTACCCAGA
 2881 CCGCCAGAGAACACAGCGAC CTTGTACAGGCTATGGCAG CACTTCAACGCGCAGGTTCAA ACAGTTGCTGATCGCGGCT TATGGAAGCACTCAGACGGC GGGCTTCAAAAGCATACTGA
 3001 CCGCGGGTACGCGCAGTACC CAGACGCGCAGGAGGCGCAC GAGCCTGGTCGCGAGGCTACG GAAGCAGCTCGACTGCGGGC TATTCAGTTCTTCTGATCGC CGGCTATGGCAGCAGCGAGA
 3121 CCGCAGGCTACGAAAGCAGC TTGACCGCGGTTACGGCAG TACGCAAAACCGCTCAGGAAA ACAGCTCGCTCACCACAGGT TACGGAAGTACTTCTACTGC GGGCTATTCAGCTCGCTCA
 3241 TCGCGGTTACGCGCAGTACG CAAACGCGCAGGCTACGAGAG CACGTTGACCGCGGTTACG GTAGTACGCAAAACCGCGCAG GAGCGCAGTATCTGGTGAC AGGTTATGGAAGTACCTCCA
 3361 CCGCGGCTATGCGAGCTCG CTGATTGCGGGTTATGGCAG CACGCAGACTCGGGGTTATG AGAGCAGTTGACCGCGGCT TACGGCAGCAGCAAAACCGC ACAGGAAAACAGCTCGCTCA
 3481 CCACCGGTTACGGAAGTACC TCCACAGCGCGCTTTGCCAG CTCGCTGATCTCCGGTTATG GCAGTACGACAGCAGCGGC TATAAAGTACCTCAGCGC CGGTTACGCGCAGTACTCAGA
 3601 CCGCAGAGTATGGAAGCTCA CTCACTGCGGGCTACGGCAG CACTGCAACGCGCGGGCAGG ACAGTTCAATTGATAGCGGCG TATGGCAGCTCCCTGACCAG CGGAATCAGAAGTTTCTGA
 3721 CCGCAGGCTATGCGCAGTACG CTGATCGCGGACTTCGCAG CGTTTTGATCGCGGTTATG GCAGTAGTCTTACATCGGGC GTTCCGACAGCTTGACTGC GGGTTATGGCAGTAAACAGA
 3841 TTGCAAGTTACGCGCAGCTCG TTGATTGACGGCCATGAAAG CATTACAGTTCGCGGAAATA AAAGCATGCTGATCGCGGC AAGGGCAGCTCGCAGACAGC AGGTTTTCCGACGACGCTGA
 3961 TTGCGGTCGCGGCGAGTGA CAACTGGCGGGTGATCGCAG CCGGTTGATTGCCGGTGCG ACAGTAAACAGACCGCGGT GACCGCAGCAAACTGCTGGC CGGTAATAACAGTTATCTGA
 4081 CTGCGCGGATAGAAGCAAA CTGACCGCGGGCATGACTG CACCTGATGCGCGGAGACC AAAGCAGATTGACCGCTGGT AAGAACAGTGTCTTGACGGC AGGCGCTCGTAGCAAACTGA
 4201 TTGCGAGTGAAGGCTCGACG CTCTCGGCTGGAGAAGACTC CATACTATTTCAGGCTCT GGGACGGGAAGAGGTACAGG CAACTGGTCGCGAGAACGGG TGAGAAGCGTGTGAGGCCG
 4321 ACATACCGTATTACGTGAAC GAAGATGACGATATTGTGCA TAAACCGCAGGAGCAGATG ACTGGATAGAGTTAAAGTAG CCGCGGTTATTCAAGCACTC CAGCACTGAATCATCGACAG
 4441 GCGCGCTCGATGGAATTC

Fig. 1 Nucleotide sequence of the genomic restriction fragment from *P. syringae* S203 which confers ice nucleation activity in *E. coli*. Nucleotides are numbered in the 5' to 3' direction. The following sites are underlined: 1-6, *Bgl*II site; 787-790, putative ribosome binding site; 798-800, first methionine codon in the long open reading frame; 4,398-4,400, termination codon for the long open reading frame; 4,453-4,458, *Eco*RI site.

Methods. Subsections of the *Bgl*II/*Eco*RI fragment were cloned into pUC8 and pUC9 (ref. 7). A range of restriction fragments was generated by partial digestion with *Sau*3A, *Hpa*II or *Taq*I, and complete digestion with *Eco*RI or *Hind*III. These fragments were cloned into the M13 mp10 and mp11 vectors¹⁰. Single-stranded DNA from M13 clones was prepared from individual plaques, the inserted DNA fragments were oriented relative to one another¹¹, and single-stranded DNAs were sequenced by the dideoxy termination method of Sanger *et al.*¹² using an M13 universal primer¹³. Gel electrophoresis was performed as described by Sanger and Coulson¹³. All information is derived from readings of both strands. Each strand was read twice, and discrepancies corrected, before comparison with the other strand.

matrices⁸. A 16-residue periodicity is present throughout the same region; the 16-letter 'words' in Fig. 2 emphasize this. In addition, a 48-residue periodicity is present in two regions, residues 208-591 and 592-959. Each line in these regions corresponds to a repeat of this order.

Have the various orders of periodicity in *inaZ* arisen by amplification or by convergent evolution under selective pressure? The codon usage pattern for serine allows us to infer evidence for the former mechanism. Two mutations are necessary to interconvert serine codons of the TCN and AGPy types, with the intermediate triplet encoding a different amino acid. Therefore, where serine is strongly conserved, its triplet will remain 'trapped' in one of the two codon types. By assuming minimum mutational distance, we calculated the most probable derivation of the consensus 48-residue repeats via amplification of a basic octapeptide (Fig. 2b). This predicts a particular codon

usage pattern which is compared with the observed pattern to test the amplification hypothesis. Serines derived from the serine at position 5 in the ancestral octapeptide should all be encoded by the same triplet type, though which one is not predicted. The same is independently true of the set of serines which occupy position 14 of the 16-mer, and of those which occupy position 7 of the 48-mer. The predicted pattern of codon usage can be seen with only one exception, in the very last octapeptide. All but one of the serines at octapeptide position 5 are encoded by the AGY triplet type; those in other positions are encoded by TCN triplets. We also examined the codon usage patterns of other amino acids, but not surprisingly these were considerably more random.

On the basis of codon usage, therefore, we can judge that the observed periodicities are unlikely to have evolved through convergence. However, we cannot distinguish between

Fig. 2 Amino-acid sequence of the *inaZ* gene. Amino acids are abbreviated as follows: A, alanine; C, cysteine; D, aspartate; E, glutamate; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine. Serines encoded by the TCN-type codon are underlined; serines encoded by the AGPy-type codon are represented in lower case. *a*, Predicted translation product of the open reading frame identified in Fig. 1. An arbitrary layout was chosen to emphasize certain features of the periodic structure. *b*, Hypothetical derivation of the observed repeat diversity during three rounds of amplification. Each dot denotes a single base change occurring between rounds of amplification. The final two 48-residue blocks correspond to two segments of the observed sequence, which consist of residues 208–591 and 592–959, respectively. The scheme shown requires the minimum number of base changes.

a	1	MNLDKALVLRTCCANNMADHCGLIWPSAGTVESTRYQSTRRHENGLVGLLW	50		
	51	GAGTSAFLSVHADARWIVCEVAVADIISLEEPGMVKVFPRAEVVHVGDRI	100		
	101	ASHFISARQADPASTSTLTTPMPTAITPTMPAVASVTLPAVEQARHEVF	150		
	151	DVASVAAAAAPVNTLPVTTQPNVQT	175		
	176	ATYGSTLSGDNHsRLI	AGYGsNETAGNHsDLI	207	
	208	AGYGsTGTAGSDsWLV	AGYGsTGTAGGdsALT	AGYGsTQTAREGsNLT	255
	256	AGYGsTGTAGSDsSLI	AGYGsTGTSGGdsSLT	AGYGsTQTAREGsNLT	303
	304	AGYGsTGTAGSDsSLI	AGYGsTGTSGGdsSLT	AGYGsTQTAREGsNLT	351
	352	AGYGsTGTAGSDsSLI	AGYGsTGTSGdsALT	AGYGsTQTAREGsNLT	399
	400	AGYGsTGTAGSDsSLI	AGYGsTGTSGdsSLT	AGYGsTQTAREGsILT	447
	448	AGYGsTGTAGVdsSLI	AGYGsTGTSGdsSLT	AGYGsTQTAREGsNLT	495
	496	AGYGsTGTAGADsSLI	AGYGsTGTSGsEsSLT	AGYGsTQTAREGsTLT	543
	544	AGYGsTGTAGADsSLI	AGYGsTGTSGsEsSLT	AGYGsTQTAREGsVLT	591
	592			SGYGsTQTAGAAsNLT	607
	608	TGYGsTGTAGHsFLI	AGYGsTGTAGHKsILT	AGYGsTQTARDGsDLI	655
	656	AGYGsTGTAGSGsSLI	AGYGsTGTASyrsmLT	AGYGsTQTAREHsDLV	703
	704	TGYGsTGTAGSsSLI	AGYGsTGTAGFKsILT	AGYGsTQTAREQTSsLV	751
	752	AGYGsTGTAGSsSLI	AGYGsTGTAGYEstLT	AGYGsTQTAREGsSLT	799
	800	TGYGsTGTAGYsSLI	AGYGsTGTAGYEstLT	AGYGsTQTAREGsDLV	847
	848	TGYGsTGTAGYsSLI	AGYGsTGTAGYEstLT	AGYGsTQTAREGsSLT	895
	896	TGYGsTGTAGFsSLI	SGYGsTGTAGYKsTLT	AGYGsTQTAREYGsSLT	943
	944	AGYGsTGTAGQDsSLI			959
	960	AGYGsSLTsGIRsFLT	AGYGsTLIAGLRsVLI	AGYGsSLTsGVRsTLT	1007
	1008	AGYGsNQIASYGsSLI	AGHsIQVAGNKsMLI	AGKGSsQTAGFRsTLI	1055
	1056	AGAGsVQLAGDRsRLI	AGHsVQLTAGDRsKLT	AGNNSYLTAGDRsKLT	1103
	1104	GGHDCITLMAGDQsRLT	AGKNSVLTAGARsKLI	GsEGSsTLsAGEDsSLI	1151
	1152	FRLWDGKRYRQLVARTGENGVEADIPYVYNEDDDIVDKPEDDDWIEVK			1200

b

AGYGsTGTAGSDsSLI AGYGsTGTSGGdsSLT AGYGsTQTAREGsNLT

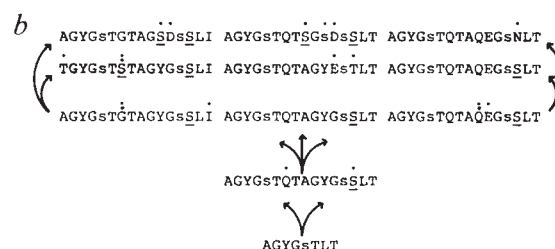
TGYGsTGTAGYsSLI AGYGsTGTAGYEstLT AGYGsTQTAREGsSLT

AGYGsTGTAGYsSLI AGYGsTGTAGYsSLT AGYGsTQTAREGsSLT

AGYGsTGTAGYsSLT

AGYGsTGTAGYsSLT

AGYGsTGT



amplification and intragenic mismatch correction as possible causes for the current structure of *inaZ*. Both phenomena are related to recombination, and both seem likely to have been involved in the evolution of this gene.

What may be the function of the repeating elements of protein structure? It is attractive to hypothesize that each element may provide an individual unit for binding water molecule(s). Their strictly conserved periodicity could provide (in at least one dimension) the regularity required for the water-binding array of an ice nucleus. A prediction of this hypothesis is that no particular part of the repeating structure will be essential for ice nucleation, although each will contribute individually to the nucleation process. To test the prediction, we isolated deletions running between *Hpa*II sites in the *inaZ* gene. Most of these deletions did not cause frameshifts and did not disrupt the 8-amino-acid periodicity, because *Hpa*II sites occur mainly at one position within the 24-base repeats. The maps of 17 *Hpa*II deletions are shown in Fig. 3; a deletion constructed with *Sal*I which causes a shift in reading frame ($\Delta 99$) is included for comparison.

The simplest statistic with which to describe the deletions is

the temperature at which nucleation frequency rises above undetectable levels. We measured this temperature by slowly cooling replicate culture aliquots, each containing $\sim 3 \times 10^8$ cells, and recording the temperatures at which the aliquots froze. The method was reproducible to within 0.3 °C and we calculated the mean 'highest nucleation point' for each deletion. The results in Table 1 (first column) show that every deletion causes a reduction in nucleation temperature, but that most of the non-frameshift deletions retain detectable activity. The amount of the reduction varies over a range of more than 8°. How are such reductions related to the nature of the mutations? Deletions close to $\Delta 124$ appear to give particularly severe deficiencies, but our sample of deletions is too diverse to permit conclusions about the importance of each level of periodicity to ice nucleation. However, it can be concluded from the results that most individual parts of the repeating structure contribute quantitatively to ice nucleation, but are qualitatively inessential. (This does not imply that all such parts could be deleted simultaneously.) This property would be predicted for a structure in which repeats individually perform identical functions as units of a water-binding array.

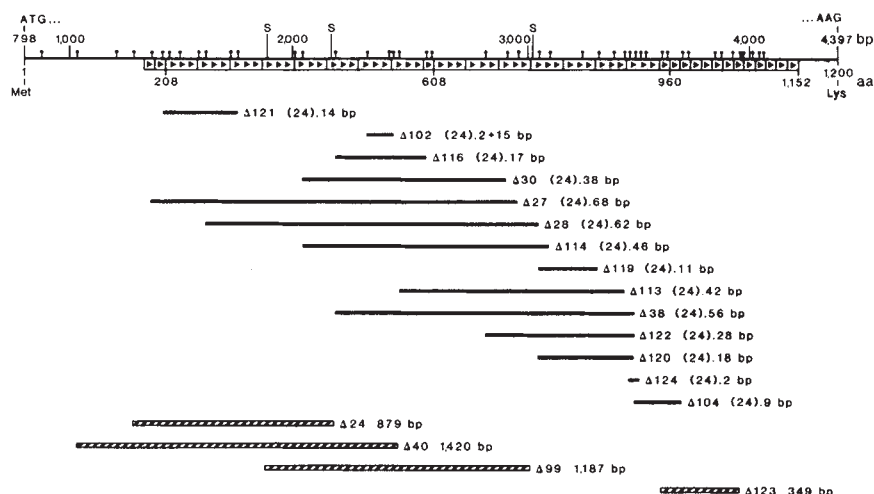


Fig. 3 Map of deletions in *inaZ*. S, *Sal*I site. Dot on stalk, *Hpa*II site. Solid bars denote deletions which do not change the reading frame of downstream sequences. Striped bars denote frameshift deletions. The number of base pairs removed is shown next to each deletion. For a non-frameshift deletion, this is shown as a multiple of the octapeptide-coding unit (24 base pairs; bp). Thus, $\Delta 124$ removes two octapeptides (=48 bp) and is therefore marked '(24).2 bp'.

Table 1 Ice nucleation characteristics of the deletions

Allele	Highest nucleation point	Nucleation frequencies per cell	
		-5.0 °C	-8.0 °C
Wild type	-3.7	1.1×10^{-2}	7.0×10^{-2}
$\Delta 121$	-4.0	1.0×10^{-3}	7.0×10^{-2}
$\Delta 102$	-5.4	NT	9.7×10^{-4}
$\Delta 116$	-6.0	NT	1.4×10^{-3}
$\Delta 30$	-4.1	1.1×10^{-7}	6.3×10^{-5}
$\Delta 27$	-9.7	NT	NT
$\Delta 28$	-7.9	NT	NT
$\Delta 114$	-9.1	NT	NT
$\Delta 119$	-10.0	NT	NT
$\Delta 113$	-5.9	NT	6.7×10^{-5}
$\Delta 38$	<-13.0	NT	NT
$\Delta 122$	<-13.0	NT	NT
$\Delta 120$	<-13.0	NT	NT
$\Delta 124$	-6.5	NT	8.0×10^{-7}
$\Delta 104$	-12.2	NT	NT
$\Delta 24$	<-13.0	NT	NT
$\Delta 40$	<-13.0	NT	NT
$\Delta 99$	<-13.0	NT	NT
$\Delta 123$	<13.0	NT	NT

We show the warmest temperatures at which ice nucleation is detectable in populations of bacteria carrying the appropriate deletions, and also the frequencies of nuclei at -5 °C and at -8 °C in these populations. NT, not tested. **Methods.** The deletion-containing plasmids (all derivatives of pRLG12), present in the *E. coli* K-12 Δ (*recA-sr1*)303 strain JC10291, were grown to stationary phase in 5-ml L-broth cultures at 24 °C with aeration. Cultures were incubated at 4 °C for 1 h before testing. Frequency measurements were performed using 10- μ l droplets from a dilution series as described by Orser *et al.*⁴. Temperature measurements were performed after diluting 100 μ l of culture into 10 ml distilled water at 0 °C; the dilutions were then cooled under observation and their nucleation temperatures recorded.

For the deletion alleles which retained activity at sufficiently warm temperatures, we measured the nucleation frequency of cultures carrying the deletion plasmids at two different temperatures, -5 °C and -8 °C. While the wild-type gene shows a frequency difference of about one order of magnitude between the two temperatures, for the deletions the difference is significantly larger; in some cases no activity is detectable at the warmer temperature whereas activity is relatively high at the cooler temperature (Table 1). This indicates that the deletions cause a change in the shape of the temperature-frequency profile, rather than a uniform reduction in frequency at all temperatures. Changes of this nature are predicted if the number of water-binding arrays is not altered, but the size of each is reduced, by deletion of octapeptides.

It has not escaped our attention that the 48-residue periodicity corresponds to a sixfold repetition of the octapeptide building block. From this one can readily construct models with symmetry to match the hexagonal structure of ice. It will be interesting to learn whether the ice nucleating protein of hornet haemolymph⁹, which has a quite different amino-acid composition, possesses a similar organization of repeated domains.

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Autoregulation of tubulin synthesis in enucleated cells

Joan M. Caron[‡], Albert L. Jones, Leslie B. Rall* & M. W. Kirschner[‡]

Intestinal Immunology Research Center, Cell Biology Section, 151E, Veterans Administration Medical Center, 4150 Clement Street, San Francisco, California 94121 and Departments of Medicine, Anatomy and † Biochemistry, University of California, San Francisco 94143, USA

* Chiron Research Laboratories, Chiron Corporation, Emeryville, California 94608, USA

The effects on tubulin messenger RNA levels and tubulin protein synthesis of treating cells with microtubule-depolymerizing drugs or directly microinjecting cells with tubulin have suggested that non-polymerized tubulin depresses its own synthesis¹⁻⁴. The precise level of this control is unclear. It has been shown that enucleated cells, termed cytoplasts, retain many properties of the original cell, including maintenance of cell shape, pinocytic activity and locomotion⁵ as well as biosynthetic activities such as protein synthesis^{6,7} and replication of cytoplasmic viruses⁸. Furthermore, cytoplasts retain most of the components of the cytoskeleton including the centrioles^{5,9}. If cytoplasmic activities alone are responsible for regulating tubulin biosynthesis, cytoplasts should contain the necessary components. To distinguish between regulation which would occur in the nucleus, that is, alterations in mRNA synthesis or modifications of the mRNA, from alterations in mRNA stability and/or translatability which would take place in the cytoplasm, we examined the autoregulation of tubulin synthesis in enucleated cells. Here, we report that enucleated mouse fibroblasts retain the ability to turn off tubulin protein synthesis in response to microtubule depolymerization, the reduction in tubulin synthesis being accompanied by a corresponding decrease in tubulin mRNA levels. Thus, transcription, processing and transport of tubulin mRNA from the nucleus are not likely to be the loci of regulation. Instead, tubulin must reduce, either directly or indirectly, the translatability of its own mRNA.

We first examined the effect of anti-microtubule drugs on the cytoplasmic cytoskeleton. Cytoplasts were prepared as described previously⁹ from mouse L929 cells and found to be $98 \pm 2\%$ free of nuclei, retaining the extensive microtubule arrays found in whole cells (Fig. 1A). Microtubules in cytoplasts and in whole cells responded similarly to colcemid and reassembled quickly after washing out the drug. Microtubule arrays in cytoplasts 4 h after enucleation were indistinguishable from microtubule patterns in 8-h cytoplasts, suggesting that enucleation did not have detrimental effects on the morphology of the microtubule cytoskeleton up to 8 h. Therefore, treatment of cytoplasts as well as whole cells with colcemid for 4 h should increase dramatically the size of the non-polymerized tubulin pool.

We next examined the ability of cytoplasts to regulate tubulin protein synthesis. Figure 2a shows an immunoprecipitate of tubulin from prelabelled cytoplasts and cells and demonstrates that addition of colcemid caused a marked inhibition of tubulin synthesis within 2.5 h. In fact, the response of cytoplasts was faster than that of whole cells (Fig. 2c). There was no change in overall levels of protein synthesis when colcemid was added. In 5 experiments with cytoplasts at 4 h after addition of colcemid, tubulin synthesis was inhibited $72 \pm 10\%$, very similar to the inhibition found in whole cells ($70 \pm 13\%$ in 7 experiments). Gel electrophoresis of total cellular extracts from cytoplasts and whole cells did not reveal any major differences in newly synthesized proteins (Fig. 2b).

However, unlike whole cells, cytoplasts did not recover the ability to synthesize tubulin protein after removing the micro-

[‡] To whom correspondence should be addressed.

Table 1 Comparison of the ability of cytoplasts and whole cells to resume β -tubulin protein synthesis on removal of colcemid

Expt 1	Relative density	% Of controls
Cytoplasts		
CON-4	99	100
COL-4	34	34
CON-6.5	100	100
COL-4/REC-2.5	34	34
Whole cells (cytochalasin B)		
CON-4	100	100
COL-4	38	38
CON-6.5	63	100
COL-4/REC-2.5	76	121
Whole cells (centrifuged)		
CON-4	84	100
COL-4	46	55
CON-6.5	100	100
COL-4/REC-2.5	65	65
Expt 2		
Cytoplasts		
CON-4	46	100
COL-4	10	22
CON-8	118	100
COL-4/REC-4	14	12
Whole cells (cytochalasin B)		
CON-4	75	100
COL-4	20	27
CON-8	120	100
COL-4/REC-4	65	54
Whole cells (centrifuged)		
CON-4	145	100
COL-4	31	21
CON-8	145	100
COL-4/REC-4	110	76

Cytoplasts were prepared as described in Fig. 1 legend in duplicate sets to determine % inhibition after addition of colcemid (first set) and recovery on removal of the drug (second set). Following a 15-min post-enucleation convalescence, half the samples in both sets were treated with 12 μ M colcemid. After 4 h, tubulin synthesis was measured in the first set of cytoplasts by short-term labelling with 35 S-methionine and immunoprecipitation of tubulin as described in Fig. 2 legend. In these experiments, immunoprecipitation was performed with the β -tubulin polyclonal antibody used in Fig. 1. After 4 h of incubation with colcemid, cytoplasts from the second set were washed four times with fresh medium and incubated for an additional 2.5 (expt 1) or 4 h (expt 2) in the absence of colcemid before lysis and immunoprecipitation. Control cytoplasts were washed along with cytoplasts which had been preincubated with colcemid. Whole cells were treated identically to cytoplasts except in one case where centrifugation was omitted and in the other case where cytochalasin B was omitted. Tubulin synthesis in whole cells was measured as described in Fig. 2 legend. Autoradiographs of newly synthesized tubulin protein were scanned (relative density) and values for controls (expt 1: CON-4, CON-6.5; expt 2: CON-4, CON-8) were set at 100%. Relative levels of tubulin synthesis after treatment with colcemid for 4 h (COL-4) and after recovery in fresh medium on removal of colcemid (expt 1: COL-4/REC-2.5; expt 2: COL-4/REC-4) were determined.

tubule-depolymerizing drug, even though microtubules were greatly reassembled (Fig. 1A). The results of two experiments are shown in Table 1. For both experiments, cytoplasts were plated in duplicate sets. The first set was used to measure per cent inhibition of tubulin synthesis after treatment with colcemid for 4 h. The second set was used to measure the ability of cytoplasts pretreated with colcemid for 4 h to resume tubulin synthesis on removal of the drug. Consistent with results from Fig. 2, tubulin synthesis in cytoplasts was greatly reduced after incubation with colcemid (66 and 78%). However, tubulin synthesis remained inhibited after removal of the drug and reformation of the microtubule arrays (66 and 88%). Two controls

were included to test the possibility that cytoplasts did not recover the ability to synthesize tubulin because of the conditions of the enucleation process. Whole cells were either incubated with cytochalasin B or centrifuged. Although there was some variability between the experiments, in both cases whole cells increased the rate of tubulin synthesis once colcemid was removed. It has been shown previously that tubulin synthesis inhibition is accompanied by a decrease in the level of tubulin mRNA. Regulation of the mRNA levels could provide a reasonable explanation for the failure of enucleated cells to reverse the tubulin inhibition. To examine this possibility we analysed the levels of α - and β -tubulin mRNA.

We found that incubation of cytoplasts with colcemid for 4 h resulted in a 47 and 62% decrease in α - and β -tubulin mRNA levels, respectively (Table 2a). A similar reduction was found in whole cells (47 and 37%). However, the level of tubulin mRNA in cytoplasts remained low after removal of the drug, whereas the level in whole cells increased significantly. Data described in Table 2a further indicate that the combined levels of α - and β -tubulin mRNAs in cytoplasts treated with colcemid for 4 h is ~20% lower than that found in whole cells. This is probably caused by continued tubulin mRNA synthesis in whole cells after microtubule depolymerization^{10,11} compared with the inability of enucleated cells to synthesize RNA.

A second experiment using Northern blot analysis confirmed the finding that treatment of cytoplasts with colcemid to depolymerize microtubules causes a reduction in tubulin mRNA levels (Table 2b). Treatment of transsected cells with colchicine has been reported to cause an increase in actin mRNA levels¹², but we have not observed such an effect. As demonstrated in Table 2b, normalization of tubulin mRNA levels against ribosomal RNA and actin mRNA gives very similar decreases, 68 and 60%, respectively, in tubulin mRNA levels. Furthermore, tubulin mRNA levels in cytoplasts remained depressed 4 h after removal of the drug (Table 2a). As the action of colcemid on microtubule physiology is rapidly and fully reversible, any putative effects on actin are also likely to be reversed quickly.

Finally, note that over a 6.5–8-h period after enucleation, we found no decrease in the level of tubulin synthesis in control cytoplasts (Table 1). However, our preliminary experiments¹⁰ indicate that the half-life of tubulin mRNA in mouse 3T6 fibroblasts is ~6 h. The same half-life for tubulin mRNA in mouse L929 cells has been reported¹³, therefore one should expect a decrease in the level of this mRNA and thus a decrease in tubulin synthesis in the control cytoplasts by 8 h post-enucleation. The fact that we did not see such a decline indicates that enucleation stabilizes mRNAs, as previously suggested by Bruno and Lucas⁷. It is interesting that in conditions that can stabilize RNAs in general, the level of tubulin mRNA can still be autoregulated by increasing soluble tubulin levels.

In conclusion, our results demonstrate that enucleated and whole cells respond very similarly to microtubule depolymerization by inhibiting tubulin synthesis. The similarity of the responses indicates that all the necessary components for such translational control reside in the cytoplasm. These results eliminate transcription, processing and transport of tubulin mRNA from the nucleus as major sites of regulation. In addition, microtubule depolymerization in enucleated and whole cells causes a decrease in the level of tubulin mRNA. However, the decreases in tubulin synthesis and mRNA levels could be two separate events. It remains to be determined whether increasing concentrations of soluble tubulin cause inhibition of translation of the mRNA and that untranscribed tubulin mRNA is unstable, or whether nonpolymerized tubulin causes degradation of its mRNA which in turn reduces the level of tubulin synthesis. Either mechanism implicates tubulin in a specific translational control as the synthesis of other proteins proceeds normally in the presence of microtubule-depolymerizing drugs. Autoregulatory mechanisms for translational control of gene expression have been well documented in two cases: gene 32 protein in bacteriophage T4 (refs 14, 15) and ribosomal proteins in *Escherichia coli*^{16,17} and yeast¹⁸. Interestingly, such

Fig. 1 Immunofluorescence micrographs of microtubules in cytoplasts (A) and whole cells (B). *a*, Controls (not drug-treated) 4 h after enucleation (A) or mock enucleation (B); *b*, treated with 12 μ M colcemid for 4 h; *c*, controls 8 h after enucleation (A) or mock enucleation (B); *d*, incubated with 12 μ M colcemid for 4 h, washed four times with medium to remove the drug and incubated with drug-free medium for 4 h.

Methods. An established cell line of mouse L929 fibroblasts was maintained in monolayer culture in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 100 μ g ml⁻¹ penicillin and 100 μ g ml⁻¹ streptomycin. Cytoplasts were prepared as described previously⁹. Briefly, glass coverslips (1.25 cm) were treated with concentrated sulphuric acid for 60 min at 60 °C, washed extensively with distilled water, rinsed in ethanol and air-dried. Coverslips were placed in Falcon 24-well tissue culture plates and sterilized by ultraviolet radiation. Cells were plated at a density which resulted in ~80% confluency after 48 h in culture. Cytoplasts were prepared by placing coverslips cell-side down in sterile 15-ml Correx tubes containing 2 ml medium with 10 μ g ml⁻¹ cytochalasin B (Sigma). After 30 min at 37 °C, 5% CO₂ cells were centrifuged at 10,000 r.p.m. for 60 min at 37 °C using a Beckman J221 centrifuge and JS13 rotor. After enucleation, cells were placed cell-side up in 24-well plates, rinsed five times with medium to remove the cytochalasin B and placed in a 37 °C, 5% CO₂ incubator for 15 min before initiation of experiments by addition of colcemid (Gibco). Although the rate of protein synthesis in cytoplasts was about fivefold lower than that of whole cells as reported by others³⁷, we found no change in the rate of protein synthesis from 15 min to 8 h after enucleation. Furthermore, by 15 min post-enucleation, cytoplasts were well spread on coverslips, resembling the morphology of nucleated cells. Therefore, a 15-min recovery time was chosen. Nucleated cells (whole cells) were plated in the same conditions as cells to be enucleated and treated identically except that incubation with cytochalasin B and centrifugation were omitted. To determine the per cent enucleation, putative cytoplasts were incubated with the nuclear stain Hoechst (described in ref. 9). In seven experiments 98 $\pm$ 2% of the cells had lost their nuclei by this enucleation procedure. Indirect immunofluorescence with an affinity-purified polyclonal rabbit anti- β -tubulin antibody² and rhodamine-labelled goat anti-rabbit antibody (Cappel) was performed as described by Karsenti *et al.*⁹

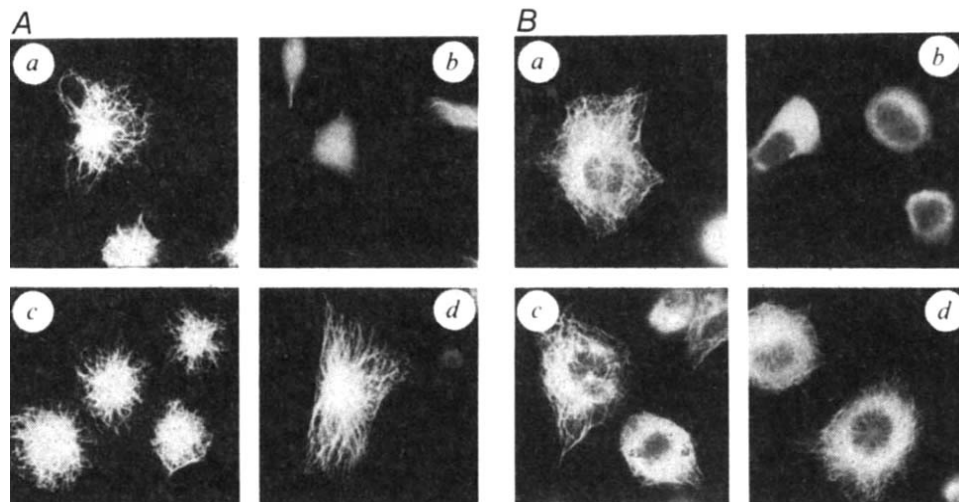


Table 2 Comparison of α - and β -tubulin mRNA levels in cytoplasts and whole cells after treatment and recovery from colcemid and after normalization against ribosomal RNA and actin mRNA

<i>a</i>	Relative density			% Of controls	
	α -Tubulin	β -tubulin	Actin	α -Tubulin	β -Tubulin
Cytoplasts					
CON-4	145	120	119	100	100
COL-4	70	41	109	53	38
CON-8	60	74	35	100	100
COL-4/REC-4	75	62	75	59	39
Whole cells					
CON-4	142	183	136	100	100
COL-4	85	130	156	53	63
CON-8	137	150	135	100	100
COL-4/REC-4	113	132	135	82	88
<i>b</i>					
Cytoplasts					
	α -Tubulin	Ribosomal	Actin	α -Tubulin/ ribosomal	α -Tubulin/ actin
CON-4	395	150	306	100	100
COL-4	210	250	410	32	40

The following procedure was used for large-scale preparation of cytoplasts required for analysis of tubulin mRNA levels. Plastic strips (2.4 $\times$ 6.4 cm) were cut from the bottom sides of Falcon tissue culture flasks with a vertical band saw, washed several times in double-distilled water, rinsed in ethanol, air-dried and sterilized by ultraviolet radiation. Cells were plated on the plastic strips and used 48 h later when ~80% confluent. Cytoplasts were prepared by placing two strips back to back per 50-ml Sorval plastic centrifuge tube containing 45 ml of medium with 10 μ g ml⁻¹ cytochalasin B, and following the procedure described for Fig. 1. After enucleation, strips containing monolayers of cytoplasts were washed five times with phosphate-buffered saline (PBS) to remove cytochalasin B and allowed to recuperate in fresh medium at 37 °C, 5% CO₂ for 15 min before initiation of experiments by addition of colcemid. Cytoplasts were incubated in the presence and absence (controls) of 12 μ M colcemid for 4 h and RNA was purified as described previously³¹. Briefly, cytoplasts were washed once in PBS and lysed with 4.0 M guanidine thiocyanate, 50 mM Na-citrate, pH 7.0, 0.5% sodium *N*-lauryl sarcosinate, 0.5% β -mercaptoethanol. Combining quadruplicate strips of cytoplasts gave a final volume of 2.7 ml. Lysates were centrifuged through 2.0 ml of 5.7 M caesium chloride, 0.1 M EDTA at 35,000 r.p.m., 25 °C for 16 h. Pelleted RNA was dissolved in sterile water, concentrated by ethanol precipitation, redissolved in sterile water and stored at -70 °C. The concentration of RNA was determined by A₂₆₀. Alternatively, cytoplasts incubated in the presence and absence of 12 μ M colcemid for 4 h were washed four times with PBS and incubated in fresh medium (no colcemid) for an additional 4 h before isolation of RNA. Nucleated cells were plated in duplicate 100-mm dishes (Falcon) and treated identically to cytoplasts except that incubation with cytochalasin B and centrifugation were omitted. *a*, Dot-blot analysis of RNA (0.5, 1.0, 2.0 μ g) from cytoplasts and whole cells was performed as described previously³². α - And β -tubulin complementary DNA³³ inserts and β -actin cDNA³⁴ insert were labelled with ³²P-dCTP by nick-translation (Cooper Biochemical). Autoradiographs were scanned (relative density) and levels of α - and β -tubulin mRNA were normalized to actin mRNA levels. Controls (CON-4, CON-8) were set at 100% and relative levels after treatment with colcemid for 4 h (COL-4) and after recovery for 4 h (COL-4/REC-4) were determined. *b*, RNA (3.5 μ g) was electrophoresed in gels of 1% agarose and 2.2 M formaldehyde³⁵, transferred to nitrocellulose³⁶, and hybridized with ³²P-labelled probes. Alternatively, to visualize ribosomal RNA, gels were stained with ethidium bromide. Band intensities after autoradiography or staining with ethidium bromide were determined by scanning densitometry.

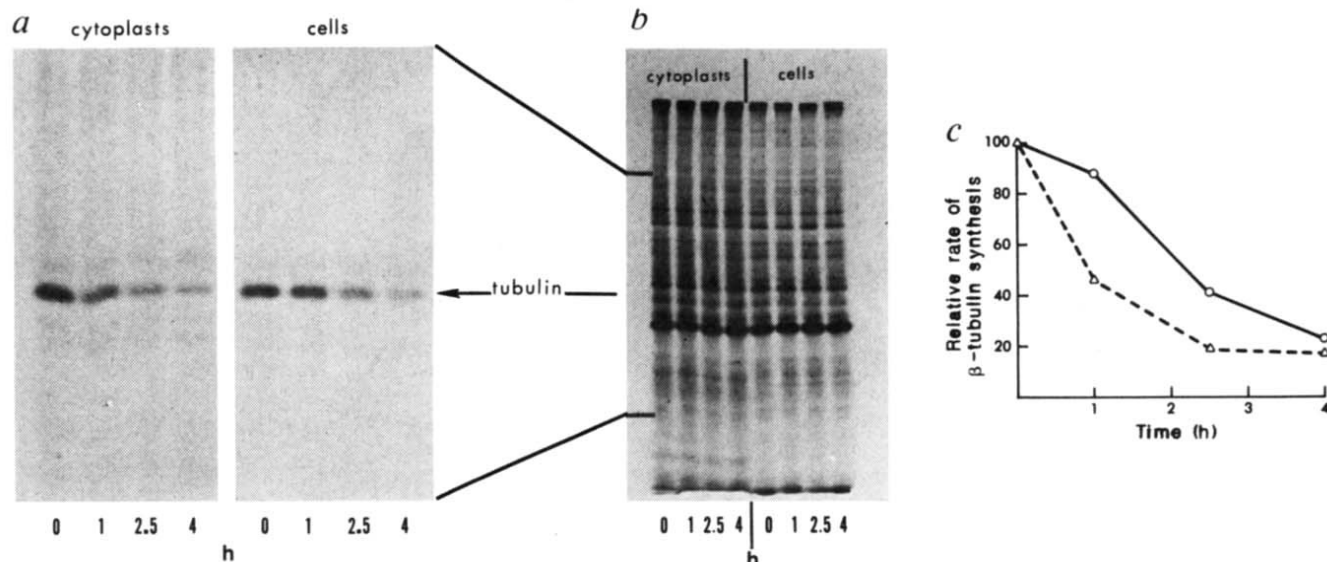


Fig. 2 Effect of microtubule depolymerization on tubulin synthesis in cytoplasts and whole cells. *a*, Autoradiograph of newly synthesized tubulin protein in cytoplasts and whole cells at several time points after addition of colcemid. *b*, Autoradiograph of newly synthesized proteins in cytoplasts and whole cells. *c*, Comparison of inhibition of tubulin synthesis in cytoplasts (triangles) and whole cells (circles) after incubation with colcemid. Band intensities of autoradiographs shown in *a* were measured by scanning densitometry using a Zenith soft laser scanning densitometer (LKB). The level of control cytoplasts and whole cells was set at 100% and relative levels at time points after addition of colcemid were determined.

Methods. Cells were cultured and enucleated as described for Fig. 1. Duplicate coverslips for each time point were processed. To measure tubulin synthesis at the same post-enucleation time in all samples (no drug, 1 h, 2.5 h, 4 h with colcemid), the following protocol was used. Following a 15-min recovery time after enucleation, colcemid was added to a final concentration of 12 μ M to the 4-h samples. After 1.5 h, colcemid was added to the 2.5-h samples. Medium was removed from all samples 1.5 h later and replaced with methionine-free medium with or without 12 μ M colcemid containing 35 S-methionine (Amersham; ~ 7 mCi 35 S-methionine was dried down using a Savant Speedvac evaporator, redissolved in 100 μ l methionine-free medium and used at a final concentration of 400 μ Ci per 175 μ l per coverslip). Cytoplasts were incubated for 1 h before the radioactive medium was replaced with 55 μ l per coverslip of lysis buffer: 25 mM Tris-HCl, pH 7.4, 0.4 M NaCl, 0.1% deoxycholate, 1% Nonidet P-40 and 0.5% SDS. Duplicates were combined at this point. Extracts were boiled for 5 min, spun at 12,000g for 3 min and the supernatant transferred to a new microfuge tube. β -Mercaptoethanol was added to 0.1% and extracts were boiled for an additional 3 min. The amount of newly synthesized tubulin protein in the extracts was determined by immunoprecipitation (150,000 trichloroacetic acid-precipitable c.p.m. per extract) as described previously^{2,38} with β -tubulin monoclonal antibody (Amersham). Immunoprecipitated proteins were analysed by SDS-gel electrophoresis on one-dimensional polyacrylamide gels³⁹ and fluorographed with En^3 Hance (NEN), dried and autoradiographed using Kodak X-Omat AR X-ray film at -70°C . Whole cells were treated identically to cytoplasts except that extracts were made with 220 μ l lysis buffer before combining duplicates. The larger volume of lysis buffer used with whole cells was chosen to make it easier to discard DNA in the extracts as its presence greatly increased background during immunoprecipitation.

autoregulation of ribosomal proteins in vertebrate cells has not been found^{19,20}.

We are now examining whether tubulin plays a direct part in its own feedback mechanism. Evidence for translational control of gene expression of various proteins has been rapidly accumulating, for example, histones²¹⁻²³ (whose synthesis, as shown for tubulin, appears to be regulated through a negative feedback mechanism), late proteins of adenovirus^{24,25}, heat shock proteins in *Drosophila*^{26,27}, light-induced proteins in *Volvox*²⁸, proinsulin in rat pancreatic islet cells²⁹ and proteins synthesized during maturation of starfish oocytes³⁰. These data indicate first, that translational control is of general importance, and second, that it can be highly varied in its molecular mechanisms. Regulation of tubulin synthesis by the degree of tubulin polymerization³ is, therefore, an important molecular mechanism.

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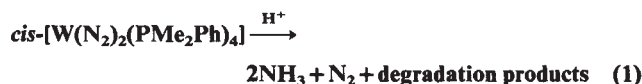
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Electrosynthesis of ammonia

Christopher J. Pickett & Jean Talarmin

AFRC Unit of Nitrogen Fixation, University of Sussex,
Brighton BN1 9RQ, UK

Since Chatt and co-workers first reported that the protolysis of the dinitrogen complex *cis*-[W(N₂)₂(PMe₂Ph)₄] gives ammonia¹,



there has existed the engaging possibility that a cyclic conversion of molecular nitrogen to ammonia might be achieved at room temperature and pressure using mediators possessing an {MP₄}_{core}, M = Mo or W. As a means of circumventing oxidative degradation of the core, coupling protonation with electronation is clearly attractive². We describe here a system in which this has been achieved for the first time and which forms the basis of an ammonia producing cycle, scheme I in Fig. 1.

The dinitrogen complex *trans*-[W(N₂)₂(Ph₂PCH₂CH₂PPh₂)₂] reacts with tosylic acid *p*-CH₃C₆H₄SO₃H·H₂O, TsOH·H₂O, in tetrahydrofuran (thf) to give the hydrazido(2-)-cation, *trans*-[W(NNH₂)TsO(Ph₂PCH₂CH₂PPh₂)₂]⁺ (A) (see scheme I, reaction (i) in Fig. 1). The cation, A, was isolated and characterized as its TsO⁻ and BPh₄⁻ salts.

Controlled-potential electrolysis of A in thf-0.2 M [NBu₄][BF₄] saturated with gaseous dinitrogen (1 atm.), at a mercury-pool cathode (*E*_{applied} = -2.6 V versus ferrocium/ferrocene, Fc⁺/Fc), consumes 2 mol electrons per mol A, and gives free NH₃ (0.22–0.24 mol per mol A) and N₂H₄ (0.01–0.02 mol per mol A) with the regeneration of the parent dinitrogen complex (0.85–0.95 mol per mol A; scheme I(ii)). Electrolysis on platinum cathodes gives substantially lower yields of the dinitrogen complex (0.6–0.7 mol per mol A).

Ammonia was identified and estimated by the indophenol test, its origination from A was established by electrolysis of the hydrazido (2-)-complex labelled with ¹⁵N and the subsequent detection of free ¹⁵NH₃ in the catholyte by ¹⁵N nuclear magnetic resonance (NMR) (δ = -315 parts per million (p.p.m.) relative to nitromethane). Electrolysis of A in thf containing 0.2 M LiClO₄ at a mercury-pool cathode gave NH₃ (0.21 mol per mol A) and N₂H₄ (0.035 mol per mol A).

Following exhaustive electrolysis on mercury, *trans*-[W(N₂)₂(Ph₂PCH₂CH₂PPh₂)₂] was the only phosphorus-containing product (singlet, δ = -94.8 p.p.m. against trimethylphosphite), and quantitative cyclic voltammetry was employed to determine the yield.

The global stoichiometry of the reduction is best represented by equation (2) in Table 1. This can be readily understood in

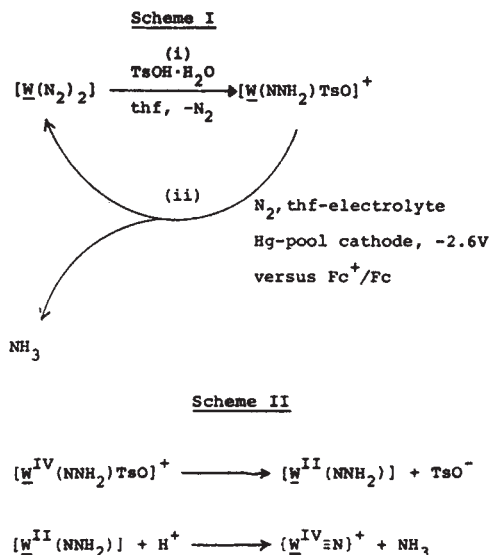


Fig. 1 Scheme I shows a basis for an ammonia-producing cycle and scheme II shows the reductive steps involved in cleavage of the W-OTs bond of A. W is *trans*-[W(Ph₂PCH₂CH₂PPh₂)₂]; H⁺ is derived from A.

terms of a series of electron- and proton-transfer reactions encompassed by the partial stoichiometries represented by equations (3)–(10) (Table 1). The partial stoichiometries are supported by the following experimental data.

Figure 2 shows the yield of NH₃ as a dimensionless function of the charge passed *q* (mol electrons per mol A). The yield of NH₃ reaches a plateau, dNH₃/d*q* = 0, near *q* = 3/4. In the NH₃-forming stage, dNH₃/d*q* is 1/3. ³¹P[¹H]-NMR spectroscopy and cyclic voltammetry show that -dA/d*q* is 4/3 and that A → 0 as *q* → 3/4. Thus no NH₃ is formed after *q* = 3/4, when A is exhausted. The maximum theoretical yield of NH₃, 0.25 mol per mol A, is in reasonable accord with the experimental yields.

Besides functioning as the primary electroactive species, A also serves as a proton source in the electrolysis. As with other related hydrazido (2-) complexes we find that A is deprotonated by bases³, including NH₃. Thus when the catholyte is purged continuously with a stream of dinitrogen and the issuing gas passed through an acid-trap (0.1 M aqueous HCl), then little NH₄⁺ is detectable in the trap before the discharge of A; it remains in the catholyte (compare equation (5) in Table 1). The converse is true at the end of electrolysis; NH₃ is purged into the trap as the intrinsic acidity of the catholyte falls.

Ramp-clamp voltammetry on a solution of A shows that intermediates are produced which have oxidation potentials appropriate to *trans*-[W(N₂H)TsO(Ph₂PCH₂CH₂PPh₂)₂] and

Table 1 The reactions involved in the electrosynthesis

$6e + \text{TsOW}^{\text{IV}}\text{NNH}_2^+ + 7\text{H}^+ + \text{N}_2 \rightarrow 2\text{NH}_4^+ + \text{TsOW}^{\text{II}}\text{N}_2\text{H}$	$d\text{NH}_3/dq = 1/3$	(3)
$\text{TsOW}^{\text{IV}}\text{NNH}_2^+ \rightleftharpoons \text{H}^+ + \text{TsOW}^{\text{II}}\text{N}_2\text{H}$		(4)
$6e + 8\text{TsOW}^{\text{IV}}\text{NNH}_2^+ \rightarrow 2\text{NH}_4^+ + 8\text{TsOW}^{\text{II}}\text{N}_2\text{H}$	$d\text{NH}_3/dA = 1/4, -dA/dq = 4.3; A \rightarrow 0$	
	$d\text{NH}_3/dq \rightarrow 0 \text{ when } q \rightarrow 3/4$	(5)
$\text{TsOW}^{\text{II}}\text{N}_2\text{H} + \text{thf} \rightleftharpoons \text{TsO}^- + (\text{thf})\text{W}^{\text{II}}\text{N}_2\text{H}^+$	} Intermediates detected	(6)
$(\text{thf})\text{W}^{\text{II}}\text{N}_2\text{H}^+ \rightleftharpoons \text{H}^+ + (\text{thf})\text{W}^0(\text{N}_2)$		(7)
$(\text{thf})\text{W}^0(\text{N}_2) + \text{N}_2 \rightleftharpoons (\text{N}_2)\text{W}^0(\text{N}_2) + \text{thf}$		(8)
$\text{NH}_4^+ \rightleftharpoons \text{NH}_3 + \text{H}^+$		(9)
$e + \text{H}^+ \rightleftharpoons \text{H}\cdot$		(10)
$16e + 8\text{TsOW}^{\text{IV}}\text{NNH}_2^+ + 9\text{N}_2 \rightarrow 2\text{NH}_3 + 8\text{W}^0(\text{N}_2)_2 + 8\text{TsO}^- + 5(\text{H}_2)$	cell current → 0 as <i>q</i> → 2	(2)

W is *trans*-[W(Ph₂PCH₂CH₂PPh₂)₂].

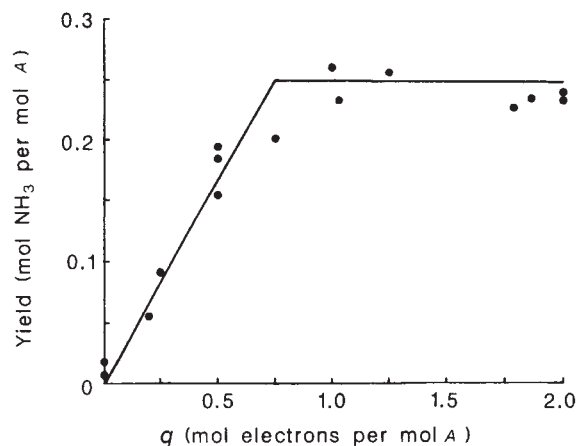


Fig. 2 The yield of NH_3 as a dimensionless function of the charge passed, q . Solid lines represent theoretical yields.

unstable *trans*- $[\text{W}(\text{N}_2)(\text{thf})(\text{Ph}_2\text{PCH}_2\text{CH}_2\text{PPh}_2)_2]$ (ref. 4). These same intermediates are generated when **A** is treated with a base in thf under argon. Under dinitrogen the thf intermediate is suppressed and complete deprotonation gives *trans*- $[\text{W}(\text{N}_2)_2(\text{Ph}_2\text{PCH}_2\text{CH}_2\text{PPh}_2)_2]$ quantitatively. A major intermediate is detected during the course of the electrolysis by $^{31}\text{P}\{^1\text{H}\}$ -NMR and ^{15}N -NMR spectroscopy and by cyclic voltammetry. The spectral data and the oxidation potential of this intermediate suggest that it is the cationic diazenido complex *trans*- $[\text{W}(\text{N}_2\text{H})(\text{thf})(\text{Ph}_2\text{PCH}_2\text{CH}_2\text{PPh}_2)_2]^+$. This species presumably arises by dissociation of TsO^- from the diazenido intermediate or by protonation of *trans*- $[\text{W}(\text{N}_2)(\text{thf})(\text{Ph}_2\text{PCH}_2\text{CH}_2\text{PPh}_2)_2]$ by **A** as it migrates from the N_2 - and proton-deficient diffusion layer into the bulk of the solution.

In the final phase of electrolysis the system is driven towards the products, free NH_3 and *trans*- $[\text{W}(\text{N}_2)_2(\text{Ph}_2\text{PCH}_2\text{CH}_2\text{PPh}_2)_2]$, by removal of protons. Electrolysis of ^2H -labelled **A**, *trans*- $[\text{W}(\text{NND}_2)\text{TsO}(\text{Ph}_2\text{PCH}_2\text{CH}_2\text{PPh}_2)_2]^+$ gave no D_2 , HD or H_2 which could be detected in the gas phase above the catholyte by mass spectroscopy. The absence of dihydrogen formation in non-aqueous media is not unfamiliar; for example, Holm and co-workers have shown that the cluster $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{3-}$ reacts in non-aqueous solvents with weak acids to give $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$ quantitatively, but no H_2 was detectable; the fate of H^+ is unknown⁵. In our system reduction of protonated thf could give rise to products other than dihydrogen: gas-liquid chromatography-mass spectroscopy of the catholyte shows that ethyloxirane and 1-methoxypropene are formed during the electrolysis, presumably as products of skeletal rearrangement of tetrahydrofuran radicals.

Whilst equations (3)–(10) (Table 1) account reasonably well for the gross features of the electrosynthesis, the crucial question is, how is electronation coupled to the protonation?

Cyclic voltammetry, repetitive-sweep open-circuit voltammetry and potential-step experiments show that the primary reductive step involves the cleavage of the $\text{W}-\text{OTs}$ bond of **A** and that this produces a highly reactive intermediate $[\text{W}^{\text{I}}\text{NNH}_2(\text{Ph}_2\text{PCH}_2\text{CH}_2\text{PPh}_2)_2]$ (Fig. 3, and scheme II, Fig. 1). Notably we find that the peak-amount ratio $i_p^{\text{ox}}/i_p^{\text{red}}$ decreases with increasing concentrations of **A**, and this is consistent with the sacrificial attack by **A** on the electrogenerated intermediate⁶.

Stable species *trans*- $[\text{M}^{\text{I}}\text{NNR}_2(\text{Ph}_2\text{PCH}_2\text{CH}_2\text{PPh}_2)_2]$ ($\text{R}_2 = -\text{CH}_2(\text{CH}_2)_{2,3}\text{CH}_2-$) can be generated by controlled-potential reduction of organohydrazido(2-) analogues of **A** under argon or by their reduction with LiBu^+ (ref. 7). They are attacked by protic acids and give imido complexes and dialkylammonium salts⁸. An analogous pathway probably accounts for formation of part of the ammonia produced in the electrosynthesis (scheme II in Fig. 1). It is not unreasonable that the remainder arises by reduction and protonation of a $\{\text{W}^{\text{IV}}\text{NH}\}$ intermediate in a like fashion.

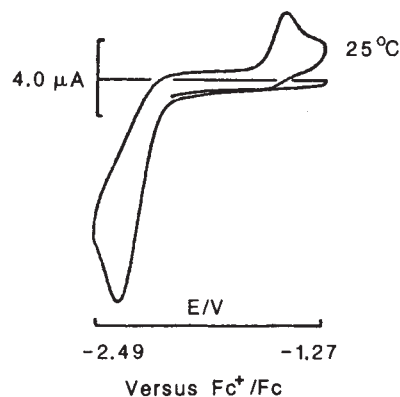


Fig. 3 Generation and detection of WNNH_2 . Scan rate 0.2 V s^{-1} at vitreous carbon electrode in $0.2 \text{ M } [\text{NBu}_4][\text{BF}_4]$. **[A]** is 1 mM .

The formation of small (0.01 – $0.02 \text{ mol per mol A}$) but nevertheless significant amounts of hydrazine occurs in the electrolyses; hydrazine is usually produced to a greater or lesser extent in all protolysis reactions of dinitrogen complexes which give NH_3 (ref. 3).

Three successive electrolysis–protonation cycles, performed on a single catholyte, established that the cyclic conversion of dinitrogen to ammonia can be achieved in one vessel, the cathode compartment of the electrolysis cell. Following the two-electron reduction of **A**, the product *trans*- $[\text{W}(\text{N}_2)_2(\text{Ph}_2\text{PCH}_2\text{CH}_2\text{PPh}_2)_2]$ was protonated *in situ* by stoichiometric addition of $\text{TsOH} \cdot \text{H}_2\text{O}$, thus regenerating **A**. The resulting catholyte was again electrolysed and the protonation–electrolysis cycle repeated once more. The yield of NH_3 after the three cycles (six electrons) was $0.73 \text{ mol per mol A}$ and of N_2H_4 $0.01 \text{ mol per mol A}$.

The conversion of molecular nitrogen to ammonia at ambient temperature and pressure by a sequence of protonation–electronation steps which involve a well-defined and conserved mononuclear core is unique. The sequence provides a chemical precedent for the hypothesis of Chatt¹ and the sophisticated model of Lowe and Thorneley⁹ which propose that biological nitrogen fixation involves successive protonation/electronation reactions of dinitrogen ligating an enzyme-bound transition-metal centre: such an enzyme centre need undergo only minimal reorganization to effect all the steps in the fixation cycle.

Further work on the electrosynthetic system might provide the basis for the development of a small-scale ammonia-producing cell. Such a cell may be of agricultural value, particularly where solar-energy collection is possible. The constraints which need be met in the design of such a cell include protection against dioxygen, a continuous supply of protons and avoidance of unproductive electronation steps (compare equations (6)–(10); Table 1). Whilst such constraints are demanding, they are not insurmountable, and they parallel those which biological nitrogen fixation has successfully overcome.

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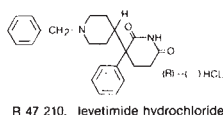
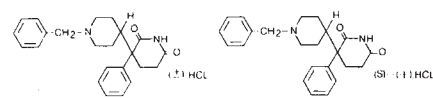
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New for the neurosciences

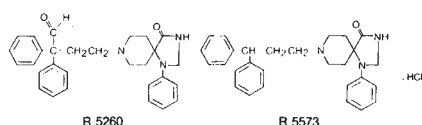
Recent developments on the neuroscience market include a range of receptor markers, two stimulators and a catecholamine assay.

• A range of specific tools for the localization and quantification of receptors in neurobiochemical research is available from Janssen Life Sciences Products Division. Serotonin (5-HT) sites can be efficiently identified with compounds R5260 and R5573, and Janssen claims the sensitivity of these two compounds is greater than any previously available. Compound R16470, the (+)-isomer of benzetimide, binds effectively to muscarinic receptors and is a good reference drug for research

Muscarinic receptors



Spirodecanone side blockers



involving the anticholinergic or muscarinic actions of drugs. Other reagents are shown in the figure above, and further details of Janssen's range for the neurobiochemist can be obtained through the reader service card.

Reader Service No. 100.

• A novel new way of determining radioactivity on autoradiograms has been developed by American Radiolabelled Chemicals, Inc. The new plastic standards contain carbon-14 and tritium in a wide range of radioactivities. These standards are mounted on microscope glass slides or coverslips. The standards are inexpensive (\$33–\$50 per set of sixteen standards) and can be used for quantitative autoradiography using carbon-14, tritium or iodine-125. For more details visit booth E-13 at the Neuroscience Meeting in Dallas.

Reader Service No. 101.

• An eight-channel computer-based stimulator has been developed by Dagan Corporation for use in electrophysiological and psychological research applications. The 9200 Omni-Pulse stimulator can be used alone to provide complex timing controls or it may be used in conjunc-



Dagan's Omni-Pulse stimulator.

tion with the 9250 stimulus isolation unit to control amplitude levels. All parameters may be preprogrammed and individual channels may be independently altered. The Omni-Pulse is capable of incrementing and discriminating single pulses or complex trains as well as controlling the voltage and current levels of the isolators.

Reader Service No. 102.

• The Model D180 cortical stimulator, now available from Digitimer, meets the requirements for electrically stimulating the human central nervous system by means of externally placed surface electrodes. The design is based on techniques developed at the National Hospital for Nervous Diseases, Queen Square, London. Externally applied electrodes connected to any ordinary electrical stimulator can give significant information about peripheral nerve disorders. Until recently, attempts to stimulate the central nervous system through the bony structure of the skull or spinal column in an effort to record at peripheral nerve sites have been unsuccessful due to unacceptable levels of pain induced in the subjects. The D180 cortical stimulator overcomes this problem by applying extremely short duration, high energy pulses. This produces activation of the motor nerve pathways beneath the bony layer without undue stimulation of the slower acting pain receptors in the surface skin layer.

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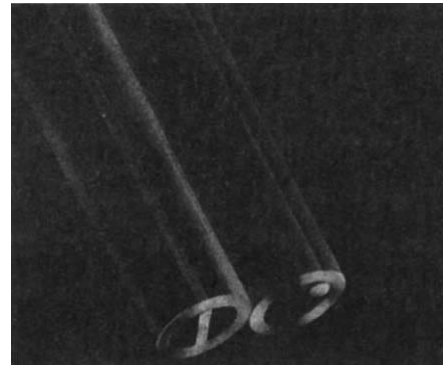


The Digitimer stimulator.

• Kwik-Fil borosilicate glass capillaries for the production of micropipette electrodes are made from high quality glass stock and are made to close dimensional tolerances to assure uniformity and reproducibility of microelectrodes. The capil-

lary microelectrodes have a filament fused to the inner wall to facilitate capillary filling. Kwik-Fil capillaries, manufactured by World Precision Instruments, are available from stock in 1.0, 1.2, 1.5 and 2.0 mm outer diameters and in one, two three, five and seven barrel configurations. Also offered are thin wall and theta style glass capillaries.

Reader Service No. 104.



Kwik-Fil capillaries from WPI.

• The Microlink Transient Capture System from Biodata is a hardware and software approach to waveform capture, storage and processing. It is designed for the measurement of fast events such as vibrations, oscillations and compressions. The system uses standard microcomputers (including the IBM PC or Apricot) and the modular Microlink hardware allows the capture of a practically unlimited number of waveforms, either simultaneously or independently. Capable also of split time-base transient capture and a variety of triggering options, the instrumentation offers 12-bit analog-to-digital conversion at rates of up to 50,000 samples per second per channel. Memory sizes of 4, 8 or 16K samples per channel are available, together with easy-to-use software.

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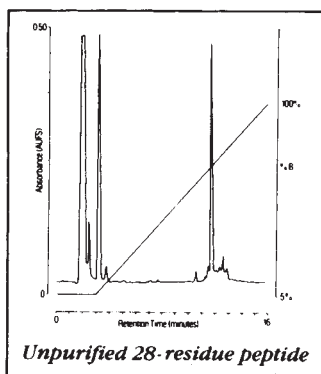
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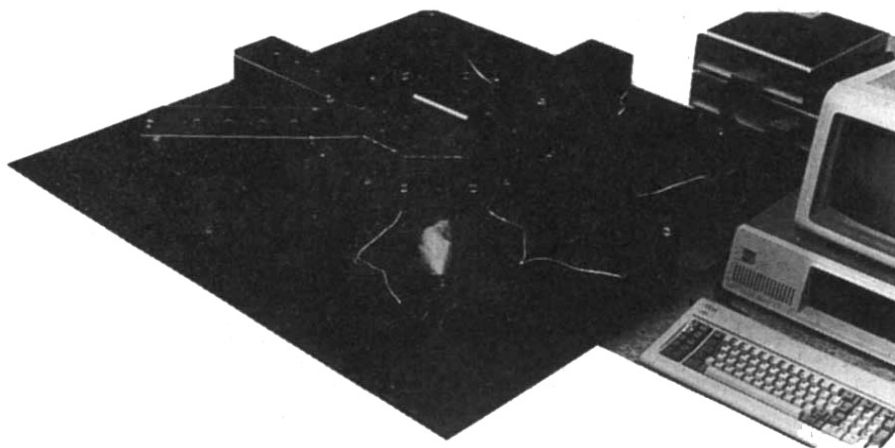
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The Radial maze from Columbus Instruments of Ohio.

• A new high performance liquid chromatography (HPLC) system for the analysis of catecholamines in plasma has been introduced by the Waters Chromatography Division of Millipore. The Waters Catecholamine System provides HPLC analysis of adrenaline, noradrenaline and dopamine at the low levels typically found in plasma. The system features electrochemical detection, programmable solvent delivery, automated sample processing and versatile data reduction. The 15-



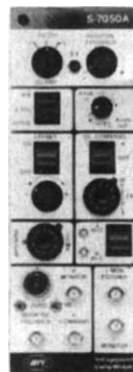
Waters' catecholamine analysis system.

minute analysis uses proven methodology and the Waters Resolve C₁₈ column for separation. Central to the system is the Waters 460 detector of high thermal mass to reduce baseline noise due to temperature change and a low 2.4 µl internal volume to minimize peak bandspread.

Reader Service No. 106.

• The S7050A whole cell/patch clamp module, part of World Precision Instruments' new S7000A microelectrode system, is capable of voltage, current and "patch" clamping single cells. Specially developed circuitry allows voltage or current to be monitored at the electrode using one of three high input impedance probes, encompassing a range of sensitivities and currents. In addition, the "patch" clamp mode can be used to perform micropolarography with a current resolution as low as 1 picoampere. This allows measurements *in vivo* on redox species, such as neurotransmitters or dissolved oxygen.

Reader Service No. 107.



• The Columbus Instruments "Radial" maze is intended for measurements of animal activity in a maze environment. The maze is made of black acrylic plastic with eight arms radiating from a central compartment. Each arm is equipped with two photo-optical infrared beam sensors at the entrance and at the end of each arm. Interruptions of light beams are fed to a 16-channel event counter interfaced to an IBM PC or Apple IIe computer and are either printed in periodic intervals, stored on the disc or both. In addition to the Radial maze, Columbus also offers the Figure Eight maze which features a shape that resembles the figure 8.

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